# eProtein Discovery™ System - Cloud Enabled - Soluble Protein Workflow

## eProtein Discovery system

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### General information

eProtein Discovery™ is the only end-to-end protein prototyping system that accelerates construct design, expression, solubility characterization and purification of target proteins in drug discovery programs. Accelerating the journey to your protein.

- Rapid protein prototyping enables progress by allowing scientists to gain awareness quickly about which proteins – and which variations of a protein – will work.
- Simultaneously screen multiple constructs and protein synthesis reagents for soluble expression, and then scale up to micrograms of recombinant protein off cartridge to test in your applications.
- Explore multiple DNA constructs, including solubility tags, polymorphisms and isoforms on the same cartridge to expand your range of accessible proteins.

## Four system components. One complete protein solution.

**Instrument:** With protein prototyping, you can draw a straight line from theory to reality, allowing you to test hypotheses more efficiently and focus on promising targets. The eProtein Discovery™ instrument puts rapid protein prototyping on your benchtop. Designed for all levels of scientist, it streamlines your workflow and grants you the ability to identify optimal DNA constructs, test expression feasibility earlier, and pursue targets with confidence. Fail fast, succeed faster!

**Software:** eProtein Discovery™ software simplifies a complex multivariate experimental design. The software sets up and simultaneously tracks 192 different combinations of DNA sequences, flank pairs and expression reagent reactions performed on eProtein Discovery™ system. AI performs highly rigorous QA checks during an experiment to ensure data quality and consistency. Informative reports are then generated and exported that you can share with your team and beyond.

**Cartridge:** Powered by digital microfluidic technology, software controlled digital signals guide the movement of droplets on the eProtein Discovery™ Cartridge surface to enable splitting, dispensing and merging of biological reagents. Pipette DNA, cell free expression reagents and purification solutions on the Cartridge and the technology will orchestrate the rest. Gain precise control of your eGene™ constructs and reagents to screen and discover optimal expressing conditions within 24 hours, accelerating target selection. A simple set-up allows anyone to run the system with minimal training.

**Reagents:** The reagents within the eProtein Discovery™ system allow you to optimize protein obtainability by characterizing and purifying different

combinations of DNA constructs and expression conditions. Our system will screen 192 different combinations in 24 hours for you to select the optimal conditions to scale up and get protein.

Our eProtein Discovery™ software will guide you in creating the panel of DNA constructs and reagents to power your experiment. Our complete reagent package includes design and ordering of DNA, simplifying your workflow.

# eProtein Discovery workflow

## Step 1: Design & Prep

Design, order and prepare linear DNA expression constructs

### Design

Input protein sequences into software and customize construct (eGene) design with pre-set fusion tags to optimize expression

### eGene prep

Prepare expression constructs with the included eGene Prep Kit

## Step 2: Load & Screen

Pipette and forget! Automated screens to determine your path to soluble, purifiable proteins

### Expression screen

Rapidly screen 192 expression conditions assessing soluble yield

24 eGenes × 8 Customizable Cell-Free Blends = 192 Expression datapoints

### Purification screen

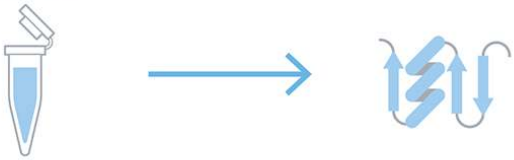
Data driven selection of 30 expression conditions for a strep purifiability assessment to determine purifiable yield

30 Purifiability data points

## Step 3: Scale up & Go!

Scale up proteins at µg- and mg-scale

### Scale up



Choose winning eGene/Cell-Free Blend combinations based on screen datapoints for scale up and purification

µl scale included and ml-scale available

µg to mg purified protein (strep)

**Total time: < 48 hours to µg of protein**

(Hands on time < 2 hours)

# eProtein Discovery Bundle

### Equipment

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| eProtein Discovery Instrument | 1 unit | Room Temperature | N1001 |  |

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### Cartridge Kit NC3006 - Consumables

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| eProtein Discovery Cartridge | 1 unit | Room Temperature | NC3006 |  |
| eProtein Discovery Cartridge Cover | 1 unit | Room Temperature | NC3012 | |

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| Base Fluid | 1 unit | Room Temperature | NC3007 |  |

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### Cartridge Reagent Kit +4°C reagent - NC3010-2

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| Strep Beads | 200 µL | +4°C | NC3010-2 |  |

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**Cartridge Reagent Kit -80°C reagents - NC3010-1 (yellow stripe on label)**

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| Cell-Free Core Reagent | 160 µL | -80°C | SC3-01 | |
| Blank Buffer | 150 µL | -80°C | SC3-02 | |
| Detector Protein* | 75 µL | -80°C | SC3-03 | |
| Universal Control* | 20 µL | -80°C | SC3-04 | |
| Complementation Control* | 20 µL | -80°C | SC3-05 |  |
| Expression Control* | 20 µL | -80°C | SC3-06 | |
| Full Workflow Control* | 20 µL | -80°C | SC3-07 | |
| Wash Buffer* | 800 µL | -80°C | SC3-08 | |
| Elution Buffer* | 50 µL | -80°C | SC3-09 | |
| AdditiveBuffer* | 50 µL | -80°C | SC3-10 | |
| PDI/GSSG Mix* | 50 µL | -80°C | SC3-11 | |

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| TRxB1* | 50 µL | -80°C | SC3-12 | |
| DNAk Mix* | 50 µL | -80°C | SC3-13 | |
| Zinc Chloride | 50 µL | -80°C | SC3-14 | |
| Calcium Chloride | 50 µL | -80°C | SC3-15 | |
| Manganese Chloride | 50 µL | -80°C | SC3-16 | |
| Cofactor Mix* | 50 µL | -80°C | SC3-17 | |
| GSSG* | 50 µL | -80°C | SC3-18 | |
| 3C protease* | 50 µL | -80°C | SC3-19 | |

Reagents must be used before the expiration date indicated on the kit box.

* Single use reagent that cannot be freeze/thawed multiple times.

### User supplied reagents

- 5 nM eGene constructs (DNA), stored at -80°C, generated using the Nuclera eGene Prep kit NC3008 or NC3009 User supplied equipment
- Magnetic particle separator (compatible with 1.5 mL microcentrifuge tubes)
- Vortexer
- Microcentrifuge

- 1.5 mL microcentrifuge tubes
- 2-20 µL 8-channel pipette
- 2-20 µL single-channel pipette
- 200 µL compatible tips

**IMPORTANT NOTE**

We recommend using a manual multichannel pipette. However, if you do not have a manual multichannel pipette and/or prefer to use an electronic pipette, the electronic pipette must be configured correctly for use with the eProtein Discovery System. Improper use of electronic pipette can result in the introduction of air bubbles during loading and can lead to a run failure. If using electronic pipette, the following settings must be enabled:

1. Disable blowout/purge function
2. Avoid high speed aspiration
3. Avoid high speed dispensing
   Please contact Technical Support (techsupport@nuclera.com) for any questions. Any run errors resulting from improper use of electronic pipettes are the responsibility of the user.

## Protein Variant Creation

The purpose of this guide is to describe a guided approach for designing protein variants, mutants, and truncated sequences.

Support users in generating variants of their protein to test on the eProtein Discovery platform and increase their chances to get quickly soluble, functional protein to use downstream in their project.

**Summary - A stepwise guided method for variant creation**

▹ **Step 1 - Identify Relevant UniProt ID** Use sequence alignment (POI sequence) or direct UniProtID input to identify the starting protein sequence and/or several close protein family members - for example isoforms and splice variants. Annotate each starting sequence with all required metadata.

▹ **Step 2 - Select Candidates** Filter isoforms, align them and flag functional or structural domains of interest.

▹ **Step 3 - Rule-based Sequence Editing I** Apply simple rule based editing for each input Candidate. Depending on the domains present, each input Candidate sequence should generate several “virtual” constructs. Remove signal peptides and propeptides, it is also often beneficial to remove transmembrane domains (TMD).

▹ **Step 4 - Rule Based Sequence Editing II - Terminal truncations** Apply simple rule based editing for each input Candidate. Consider modifications around functional domains of interest, for example removing disordered or unnecessary domains. eProtein Discovery™ System User Guide 9

▹ **Step 5 - Check for other known stable domains (NMR, X-Ray)** Identify other important regions and create relevant variants.

▹ **Step 6 - Compile final list of variant Candidates for a POI**

**Details - A stepwise guided method for variant creation**

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| 1 | Identify Relevant Uniprot ID | Sequence or Uniprot ID | Annotated Uniprot sequences | • Identify relevant isoform, canonical isoforms, orthologs, align.<br>• Identify critical domains, Uniprot, Expasy (Structural, functional, etc.) |
| 2 | Select Initial Candidates | Annotated Uniprot sequences | Isoforms and important domains flagged | • Identify relevant isoforms, canonical iso forms, orthologs, align<br>• Identify |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | critical domains, Uniprot, Expasy (Structural, functional, etc.) |
| 3 | Combine starting list | Seqs from steps 1 and 2 | List of input Candidates | Combine lists 1 and 2 |
| 4 | Rule-based Sequence Editing I - identify domains of interest | List of input Candidates | List of Child Candidates 1 Edited sequences named appropriately - rules applied see operations.<br><br>A Child Candidate is a sequence derived from an Initial | • If present remove signal peptide from N-terminus If present remove pro-peptide from N-terminus or C-terminus<br>• If 1 TMD present remove domain * If >1 TMD present discard |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | Candidate by applying Rule-based Editing - Step 4 | Candidate sequence<br>• If N-term TMD truncate after TMD (e.g. aa directly after TMD)<br>• If C-term TMD truncate before TMD (e.g. aa directly before TMD) |
| 5 | Rule Based Sequence Editing II - truncations | List of input candidates (Step 3)<br>+<br>List of Child Candidates (step 4) | | • Consider N-terminal truncation to leave the domain of interest. Users may add up to 10 aa upstream.<br>• Consider C-terminal truncation to |

| Step | Title | Input | Output | Operations |
| --- | --- | --- | --- | --- |
| | | | | leave the domain of interest. Users may add up to 10 aa downstream.<br>• Consider both N- and C-terminal truncations to leave the domain of interest. Users may add up to 10 aa upstream or downstream.<br>• Consider truncating to remove disordered regions around functional domain of interest<br>• If available |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | use structure information (X-ray, NMR, AlphaFold) to guide truncation sites |
| 6 | Compile final Screening Candidates | List of input candidates (Step 3)<br>+<br>List of Child Candidates (step 4)<br>+<br>List of Child Candidates (step 5) | | • Consider your downstream needs (e.g. activity assay, binding assay, structural investigation)<br>• Consider your cartridge format (e.g. a FlexiVariant™ or Solubility tag screen<br>• Consider your cartridge budget (e.g. do you plan on running |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | one or more cartridges within this campaign)<br>• Consider Alphafold scoring results |

# Additive Selection Guide

Depending on the protein of interest, the presence of additives may be needed to optimize the expression.
The decision tree in Figure 2 illustrates the Additive options based on protein requirements.

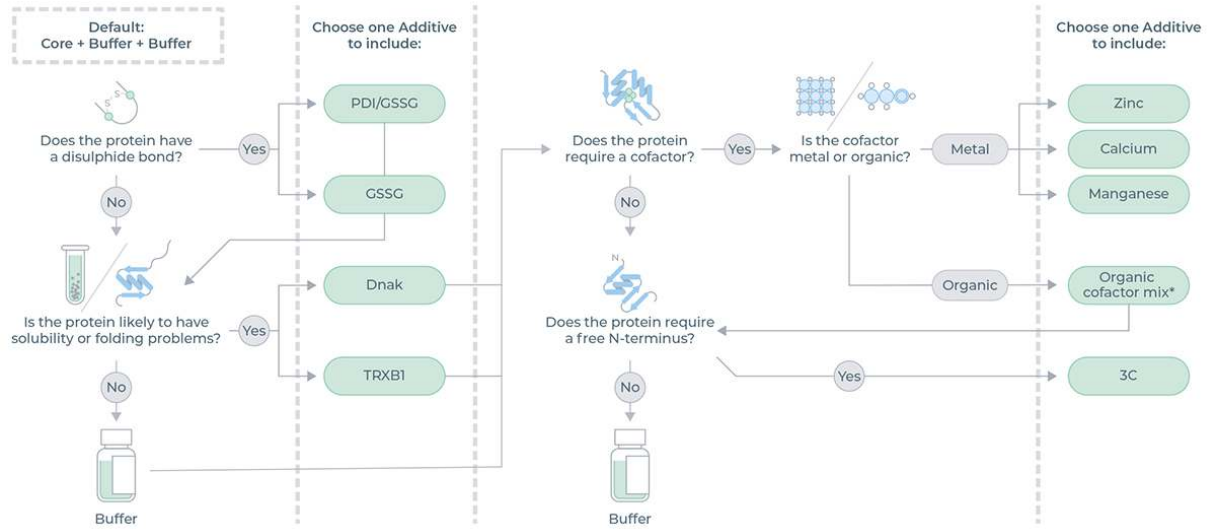



**Figure 2: Decision tree which illustrates the Additive options based on protein**

**requirements. *Mix of NAD, acetyl-CoA, FAD, SAM**

The additives supplied in the Cartridge Reagent Kit NC3010 and their descriptions are listed in Table 1.

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| Additive buffer | HEPES buffer pH 7.5 and surfactant | CFPS reaction buffer, dilution normalization |
| PDI + GSSG Mix | Protein disulfide isomerase and oxidized glutathione | Chaperone and redox modification to oxidizing environment to support disulfide bond formation |
| TrxB1 | Thioredoxin reductase | Protects proteins from oxidative aggregation and inactivation and acts as a reductase in redox regulation |
| DnaK Mix | Chaperone | DnaK mix Chaperone mix to support folding and prevent aggregation |
| Zinc chloride | Zinc chloride solution | Cofactor that can be required for folding, stability, or activity |
| Calcium chloride | Calcium chloride solution | Cofactor that can be required for compaction, folding, stabilization, or activity |

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| Manganese chloride | Manganese chloride solution | Cofactor for metalloenzymes for structure and activity |
| Cofactor Mix | Mix of NAD, acetyl CoA, FAD, SAM, and PLP | Cofactors that assist in folding, stability and activity |
| GSSG | Oxidized glutathione | Redox modification to oxidizing environment |
| 3C protease | 3C protease solution | Protease to cleave off the N-terminal solubility tag at the specific aminoacid sequence (LEVLFQ/GP) |

**Table 1: List of Additives supplied in the Cartridge Reagent Kit NC3010.**

# eProtein Discovery Software

### Intended Use

The eProtein Discovery Software, Nuclera's cloud-based software, supports the user in the design and execution of combinatorial protein expression experiments on the eProtein Discovery platform.

### Software Updates

▹ Automatic software updates are carried out when instrument is on and not in use with a message displayed on screen.
▹ If needed, automatic update settings and scheduling can be changed in

the settings of the instrument (accessible from the side menu). It is recommended to check the time zone is correctly selected.

**On our fully integrated eProtein Discovery system the user can record:**

▹ Sequences of interest.
▹ Constructs compatible with the Nuclera technology.
▹ Recipes for constructs expression and scale-up.
▹ Record a specific eGene construct (DNA) containing the sequences of the proteins of interest and the selection of the eProtein expression conditions. For the design of protein variants, mutants, and truncated sequences, it is recommended to follow the eProtein Discovery Guide for Protein Variant Creation.

More information about the eProtein Discovery™ cloud-enabled platform is available in the appendix section of the user guide.

## Intended Target User Group

The eProtein Discovery Software is intended to be used by staff trained to run experiments on the eProtein Discovery Instrument.

**How to access your service**

**Software Requirements:**

Our cloud software works on the major browsers (other browsers may also work), without the need to download or install additional packages:
▹ Google Chrome
▹ Mozilla Firefox
▹ Microsoft Edge

**For correct behavior in eProtein Discovery Cloud account, check the following:**

▹ Enable cookies. Make sure cookies are enabled in the browser so all parts

of our application work as expected.
▹ JavaScript must be enabled in the browser.
▹ Set the screen resolution Minimum screen resolution to 1024 x 768. A higher resolution is recommended.
▹ Check the web browser for add-ons. In some cases, browser add-ons, extensions, ad blockers, or plugins can interfere with our application's functionality.
▹ Consider disabling these extras or try a browser without them.

**Required Files and Other Information**

**Files and information needed for the analysis:**
▹ A valid amino acid sequence copied as text.
▹ A valid DNA sequence copied as text.

**Steps for Access**

**The following steps are required to access our service**

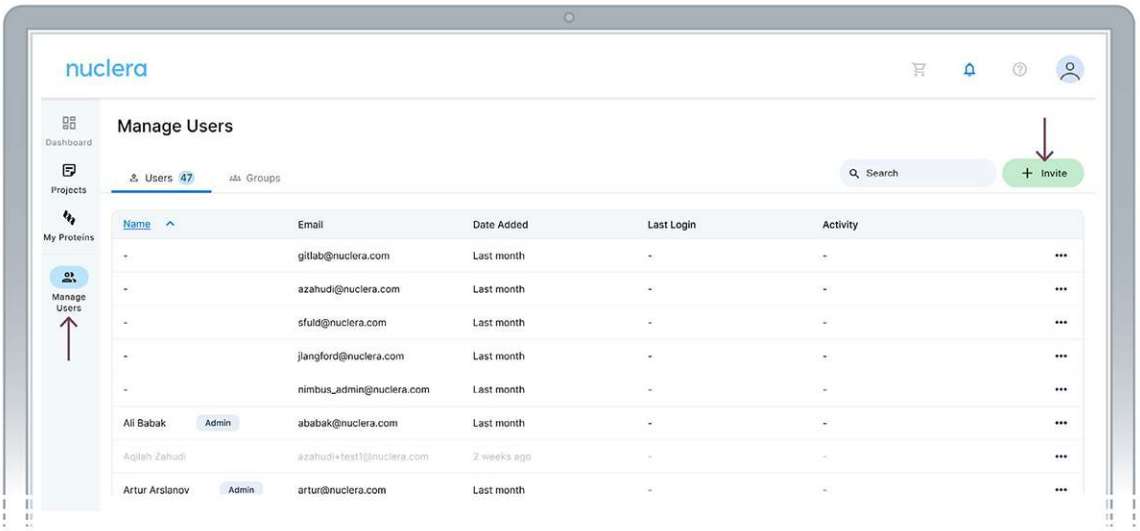
1. The first Administrator user is created by Nuclera.
2. The administrator user will log in to the platform and create projects.
3. The administrator user can invite other users and assign them to projects.
4. Non-administrator users will add their details, create password and an instrument access pin at first log in.
5. The admin users can give other users administrator privileges.
6. The admin users can deactivate non-administrator users.
7. After first login users can register proteins and create experiments in their projects

Steps 1-3 are required for first time login. Step 4 is self-service for all users. Steps 6-7 are part of everyday activity on the platform. Steps 2, 3, & 7 are

described in detailed below.

### Add a new user - Administrator

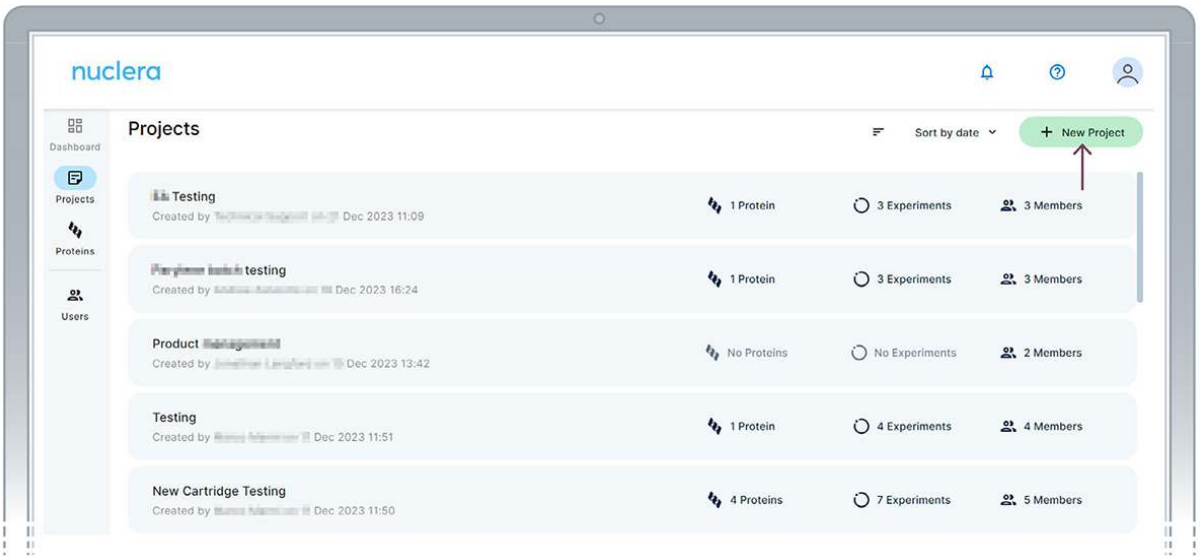
1. From the Manage Users page click the **[Invite]** button.
2. Fill the email address of the invitee and click **[Send]**.
3. The invitee will receive an email with instructions.




### Set up a project - Administrator

In the eProtein Discovery Software portal, users can create a new project or select from an existing project.

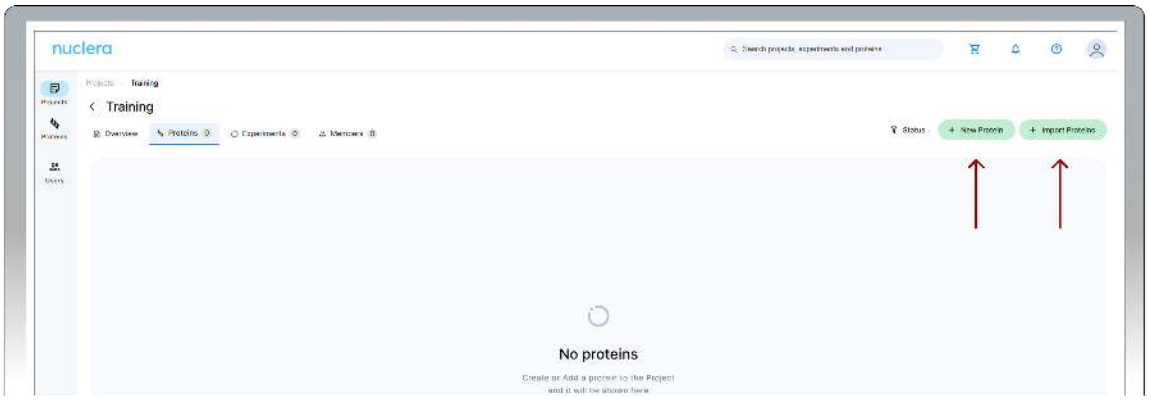
To select an existing project, click on one of the existing projects listed on the screen. To create a new project, click on the **[New Project]** button located on the top right corner of the screen.



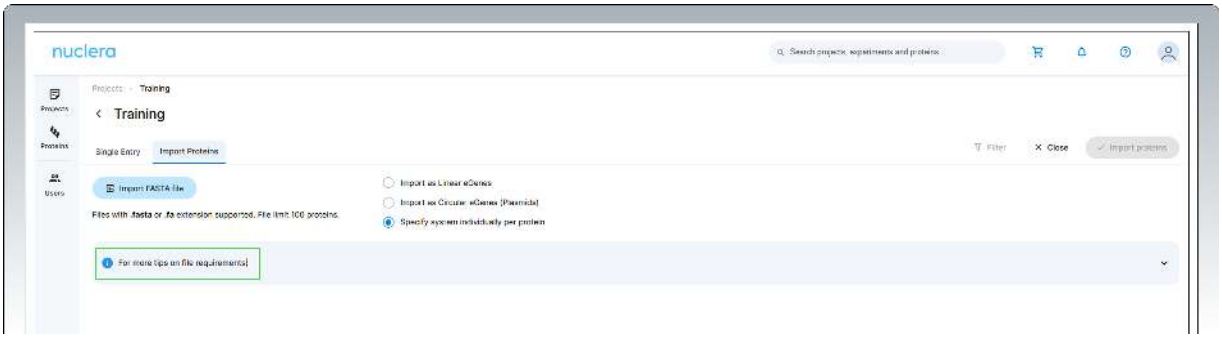

## Register a protein sequence - Any user

Once a project is created, you can navigate through it.

1. Select the **[Proteins]** tab and click the **[+ New Protein]** button.
   Bulk protein sequences can be imported from a FASTA file by clicking on the **[+ Import Proteins]** button. This feature is particularly beneficial for users looking to import 24 sequences or more at once.




For more information about the **"Import Proteins"** feature, click on the i blue symbol



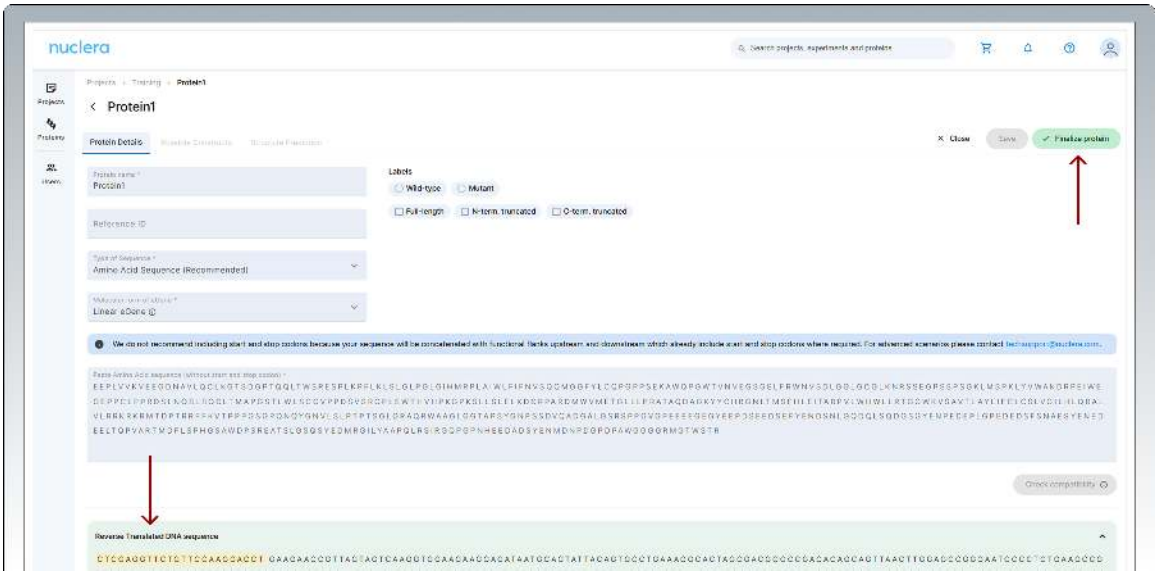

2. Add a name for your sequence and a reference ID (optional). Select the labels that apply to your protein and add any notes. Select the molecular form of the eGene constructs you want to use, Linear eGene or Circular eGene. Select type of sequence you are submitting, amino acid or DNA. Copy and paste the amino acid or a DNA sequence into the input box. Click on **[Check Compatibility]** and wait until all the tests have been performed.


3. If you have uploaded an amino acid sequence, the sequence will be codon optimized and converted to a compatible DNA sequence. For linear eGene construct format, adaptor sequences will also be added to the 5’ and 3’ end. These adaptor sequences will serve as primer annealing points during the PCR reaction to expand each construct with the appropriate fusion tags.

4. The software will perform DNA sequence compatibility checks to ensure that there are no conflicting sequences that can impact DNA synthesis.

5. Protein expression compatibility checks will also be performed in the background to detect transmembrane domains, disorder regions or the presence of start and stop codons. If any expression incompatibility is detected, a warning signal will be displayed. Users can still proceed at risk or return to modify the sequence.

6. If you are happy with the sequence, press the [Finalize protein] button located at the top right corner of the screen.




7. Once finalized, the protein or DNA sequence cannot be modified.

8. The translated DNA sequence can be copied and gene fragment ordered from Nuclera's recommended DNA synthesis vendor.

## How to order your Circular eGene™ constructs

Following the steps below, you can order your Circular eGene constructs directly from our list of approved vendors:

- GenScript®
- GeneArt®

1. **If this is your first time ordering from one of our approved vendors:**

- Contact Nuclera Technical support at: techsupport@nuclera.com requesting to access selected vendor (GeneArt® or GenScript®) details to purchase Circular eGene.
- Download the appropriate Circular eGene constructs order form and fill it in with the required information and Circular eGene construct sequences you wish to purchase.
    - GeneArt® order form
    - GenScript® order form
- Send the correct filled in form to the selected vendor once you have been contacted by the vendor directly. **Note - You must contact Nuclera Technical support every time you wish to order from a new Nuclera-approved vendor you have not ordered from before.**

2. If you have already ordered Circular eGene construct sequences from the selected vendor:

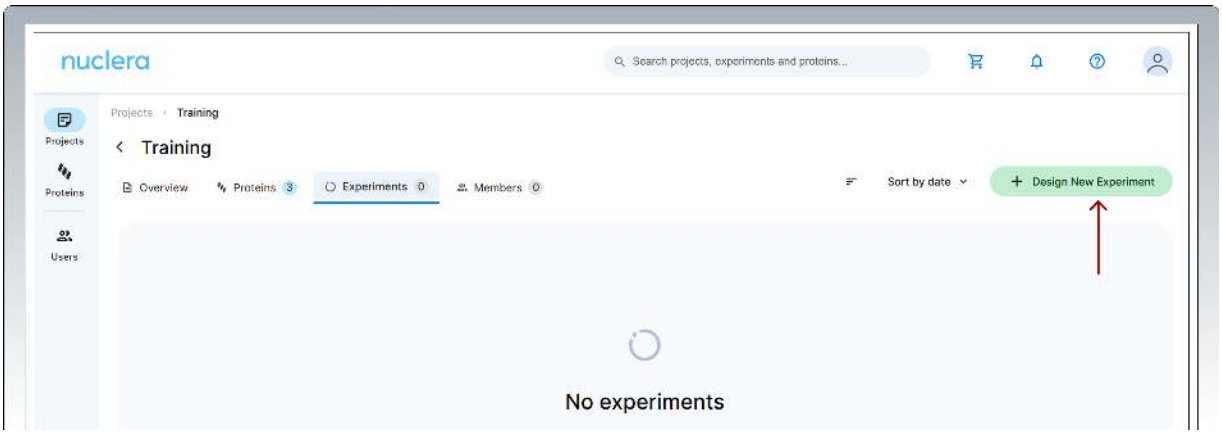
- Download the Circular eGene construct order from GeneArt® or GenScript® and fill it in with the required information and Circular eGene construct sequences you wish to purchase.
- Send the filled in form to the selected vendor to request a quote.

# Design an experiment - Any user

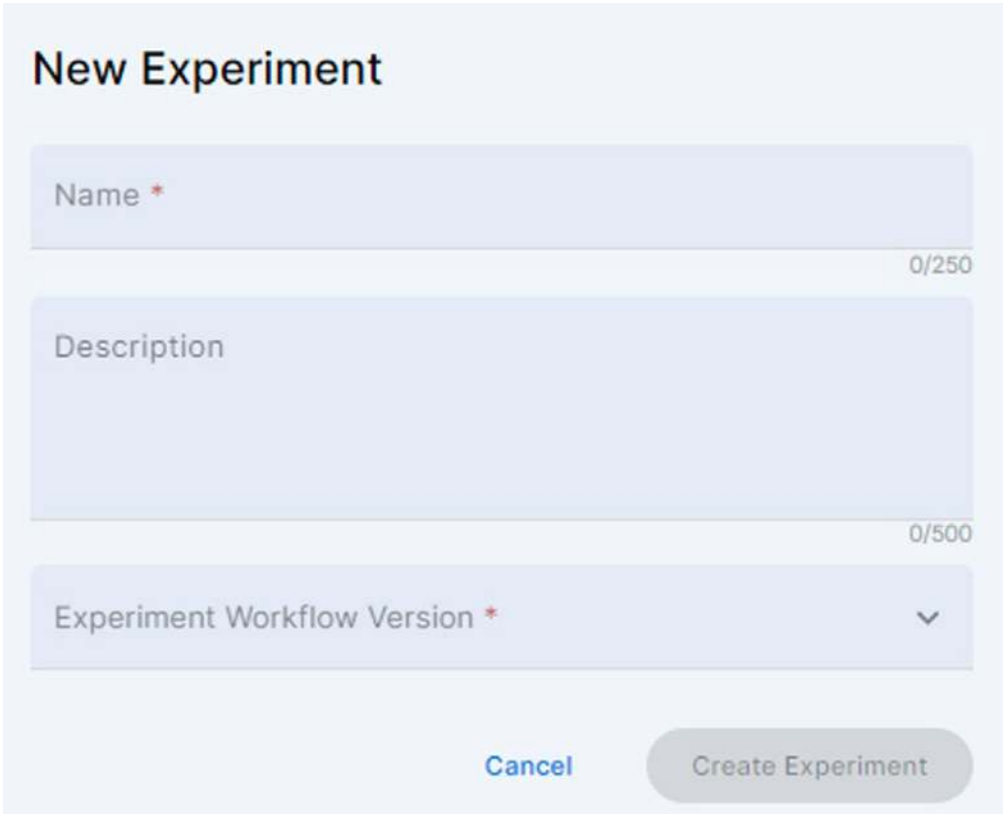
### Expression Screen

1. After registering your protein(s), you can move forward to design your experiment.

2. Select the Experiments tab and click on the **[Design New Experiment]** button.




3. Enter the name of the new experiment, add a short text description and select the eProtein Discovery Screen Experiment workflow. Click the activated **[Create Experiment]** button

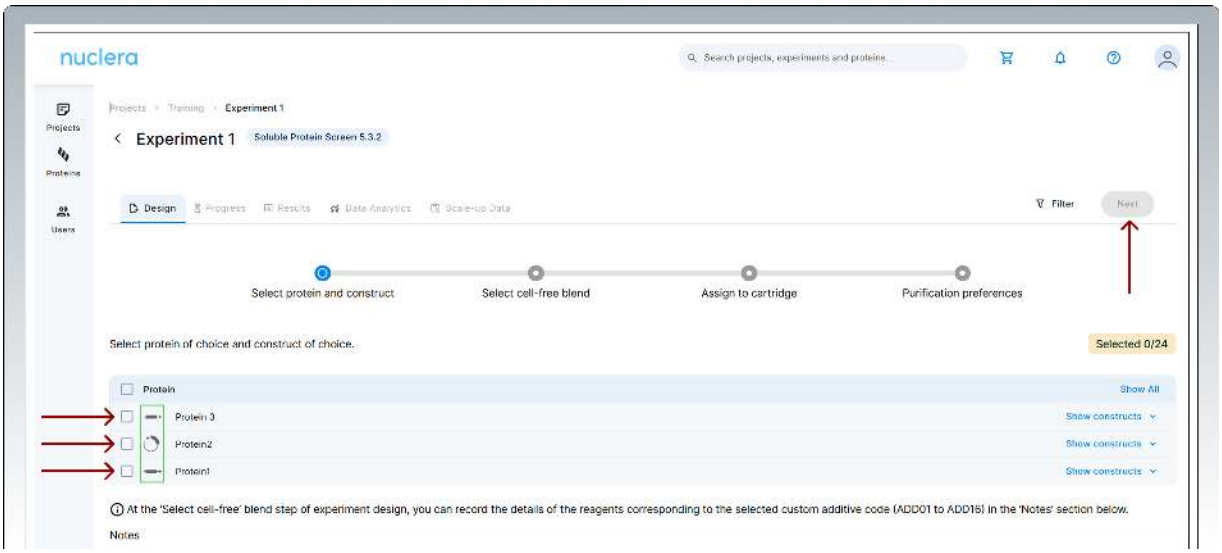


**NOTE**

If the version of the Workflow is not compatible with the current version of the Instrument Software, a warning message is displayed, inviting the user to update the Instrument Software.
**Note** On the instrument, an incompatible experiment will appear with a warning sign inviting the user to update the Instrument software.

4. Select 24 DNA constructs to assign to a cartridge. Once a desired number of constructs are selected, click on **[Next]**. The molecular form of your eGene constructs is shown next to the name of the protein chosen (green rectangle).

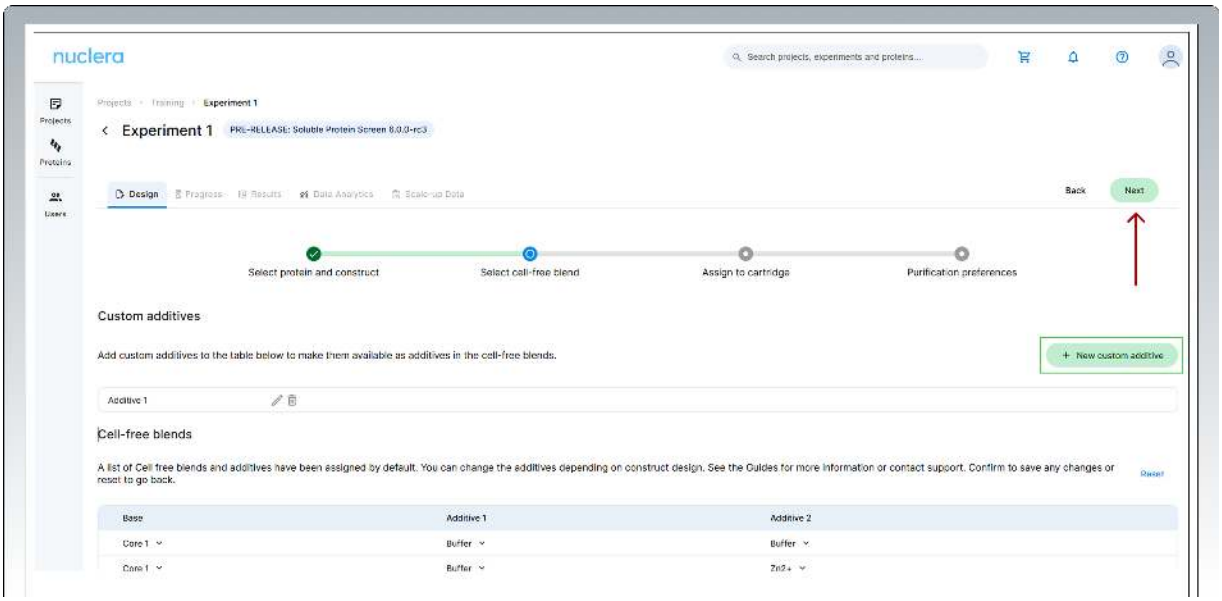
**Note:** You can load the same construct (duplicate) in two ports if required by your experimental design.




5. Select expression conditions by adding two additives to the Cell-free Core Reagent – The “Additive selection guide” section of this document will help you to select additives. If you are undecided on the additive combinations to use, go with the default list of high performing Cell-free Blends pre-selected on the screen. Click **[Next]** to proceed.

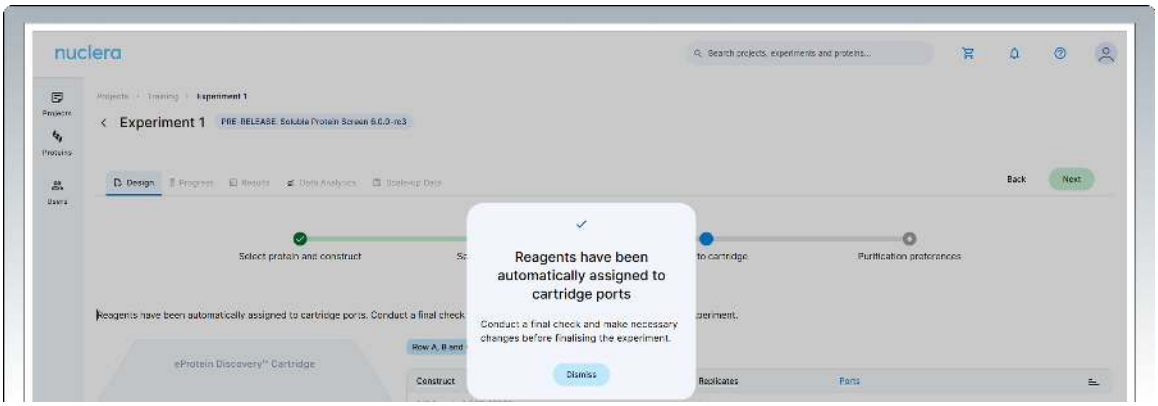
The eProtein Discovery™ system also provides the flexibility to incorporate custom additives into expression and purification workflows, enabling users to tailor conditions for unique protein targets. To ensure optimal performance and minimize risks, please consult the Compatibility List at https://info.nuclera.com/manual-custom-additives-chemical-compatibility-list.html, which provides detailed guidelines on compatible additives and their maximum allowed concentration. This resource serves as a valuable reference to help you achieve optimal results when working with custom additives. Refer to this before experimenting with custom additives or contact Technical Support if you require more guidance. Custom Additives can be shown in the experiment design by clicking on the **[New custom additive]** box. Up to 20 characters can be used for the definition of the custom additive.

> **NOTE**
>
> An additive can be selected twice to enhance a specific condition.

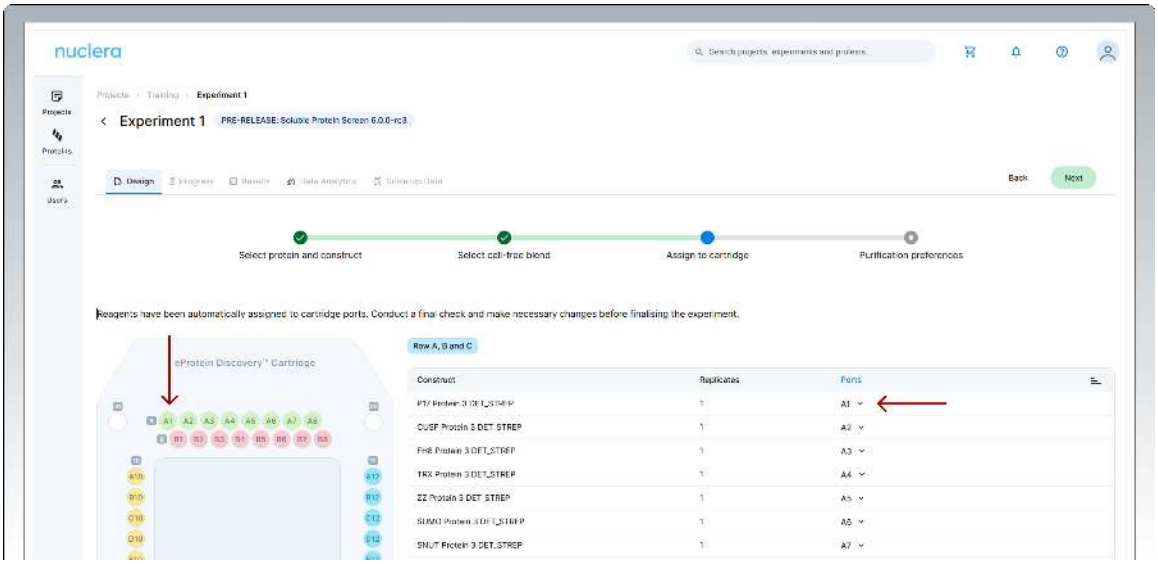



6. All samples and reagents are now assigned to a specific port on the

cartridge.

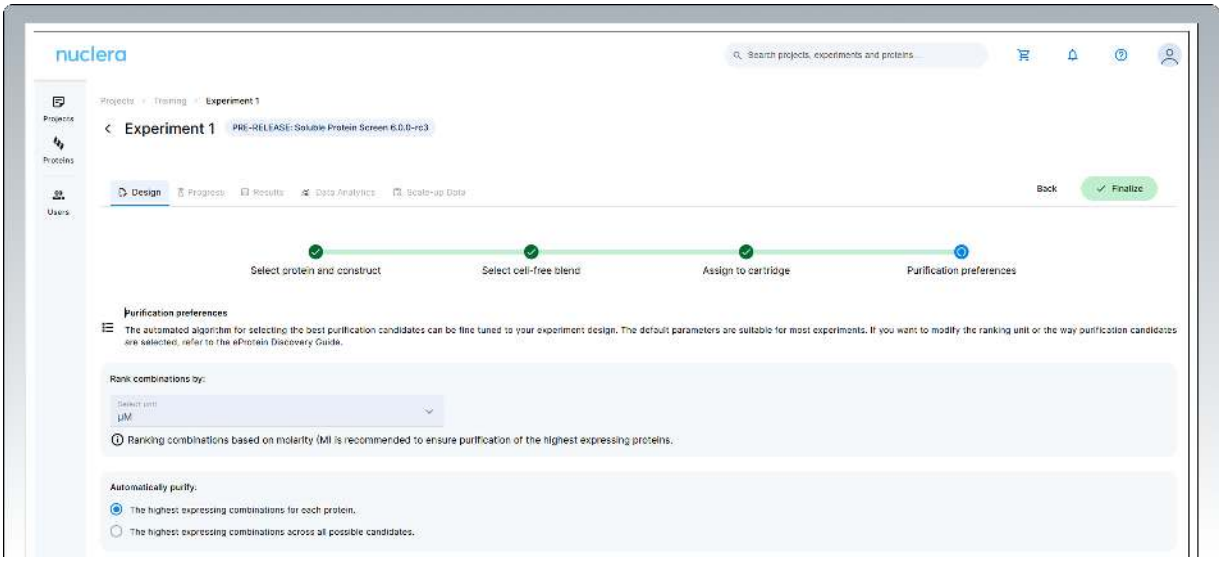



7. Review & Correct sample allocation – In the final step you have the opportunity to inspect and potentially change port location for DNA samples and Cell-free Blends. Drag & drop in the interactive cartridge map or use the port menu available to every sample. Press the **[Next]** button to proceed.




## Purification Screen

In the following step you can modify the parameters the instrument will use to select the best expression conditions for purification. The default variables are pre-selected and are suitable for the majority of experiments.




**The default algorithm works as follows:**

▹ In total, 30 expression conditions are selected for the purification screen.

▹ The selection can be done using either molar concentration (µM), recommended, or mass concentration (mg/mL) depending on the purification preferences.

▹ If you are expressing 3 POI on the cartridge (3 Proteins-of-interest against a 8 fusion tags panel), the instrument will automatically select the 10 highest expressing combinations from each construct so all the constructs are represented in the purification screen (3x10).

▹ If you are expressing 6 POI (6 POI x4 tags) the instrument will select the 5 highest expressing combinations from each protein (6x5).

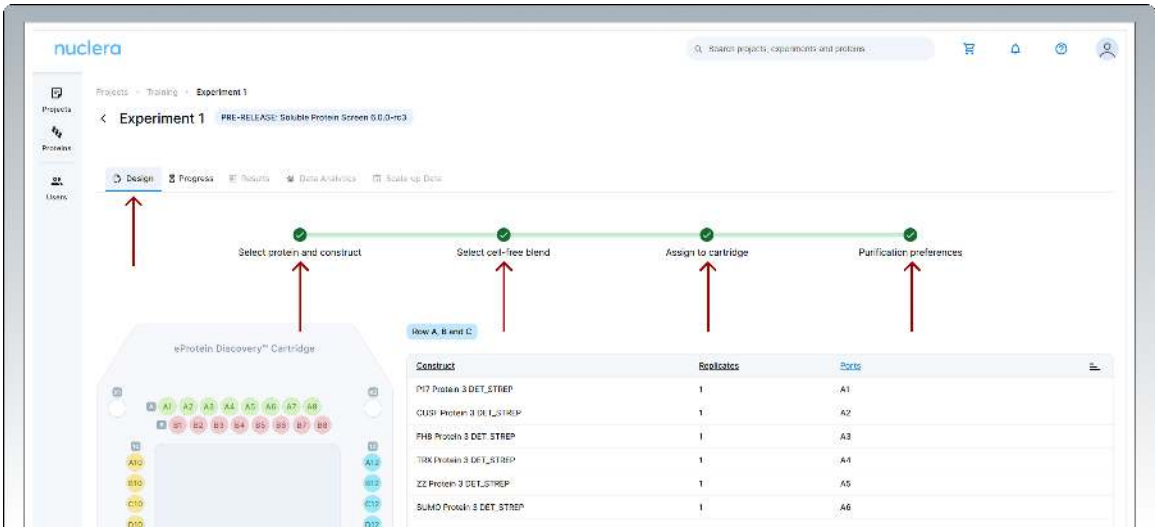
As a user you can alter the behavior of the instrument and select the best expression experiment across all 192 different conditions.

The ranking can be performed using different units, µM or mg/mL.

> **IMPORTANT NOTE**
>
> In that scenario you might end up with some of your protein

> constructs **NOT** represented in the purification.

1. Press the **[Next]** button to proceed. Confirm the statements presented by ticking the boxes. If you cannot confirm these four statements we recommend you keep the experiment in draft status (choose **[Cancel]**).
2. Press the **[Finalize]** button to proceed. Your experiment is now available on the instrument.
   **Note:** Once finalized, the experimental design cannot be changed.
3. A summary of your experiment is now available. You can toggle and review the various aspects of your experiments by selecting the Design tab and clicking any of the **[four green circles]** in the design page.




# Preparation of the eProtein Discovery reagents

The preparation of the reagents takes about 1 hour.

### Connect the vial of base fluid to the pump module

▹ In anticipation of starting a new experiment, take a fresh vial of base fluid, open it, and connect it to the left holder on the eProtein Discovery instrument pump module (Figures 3 and 4).

**IMPORTANT NOTE**

It is important to equilibrate the base fluid with the lab atmosphere prior to use. This is to prevent outgassing of the base fluid during the run, as air bubbles can interfere with the droplet movement. **We recommend attaching the base fluid to the instrument the day before you will perform the run. An acceptable alternative is to incubate the uncapped base fluid at 30°C / 86°F for 1 hour**.

▹ Connect the waste container, empty, to the right holder of the pump module (Figures 3 and 4).

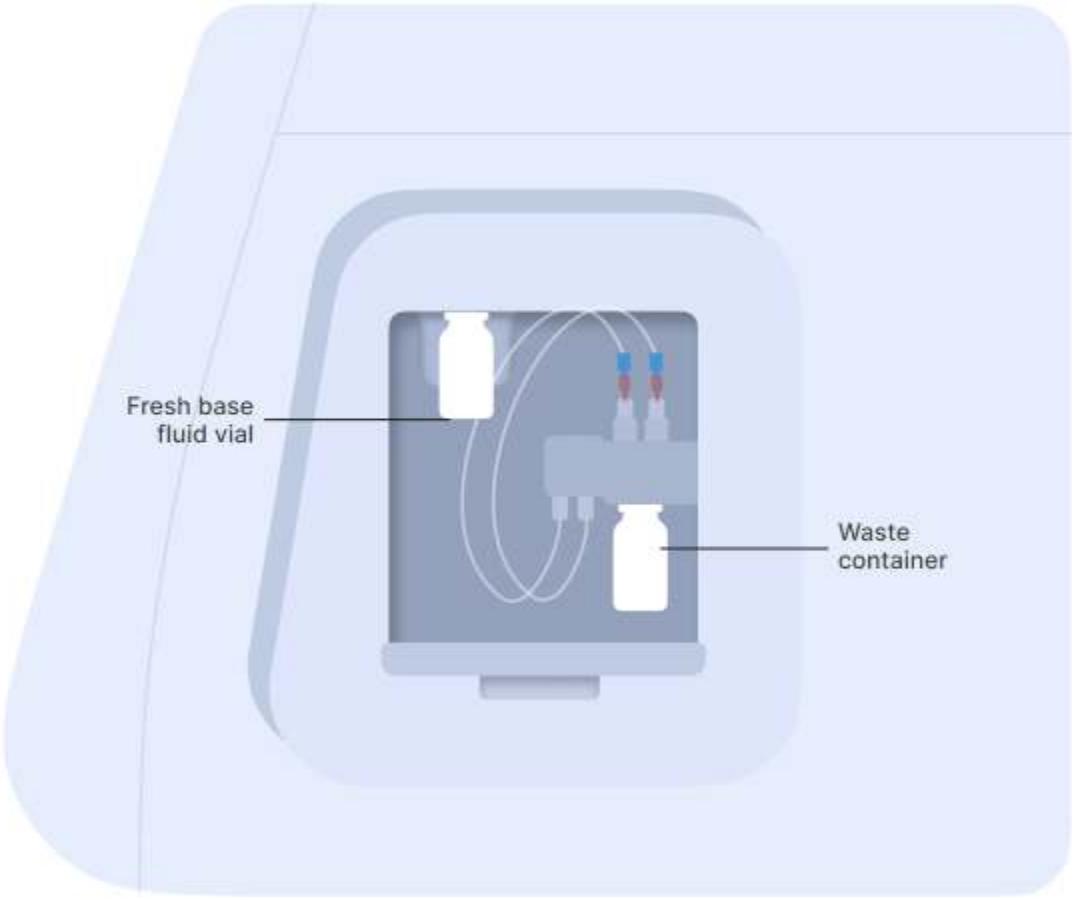



**Figure 3: Vial of base fluid and the waste container connected to the pump as shown on the screen**

[1]

[2]

**Figure 4: Connection of the empty waste container [1] and the vial of base fluid [2] to the pump**

## Prepare the transfer plate

After connecting the base fluid to the holder on the instrument, take all the reagents out of the freezer.

The eProtein Discovery reagents need to be prepared and loaded onto a 96 well transfer plate following the layout and volumes in Figure 5 and Table 2.

**Note:** It is critical to follow this layout exactly because it determines how the reagents are dispensed in the eProtein Discovery cartridge.

**IMPORTANT NOTE**

It is critical not to leave any port empty. If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit, **not with water.**

**Tip:** Empty ports can be used for duplicates.

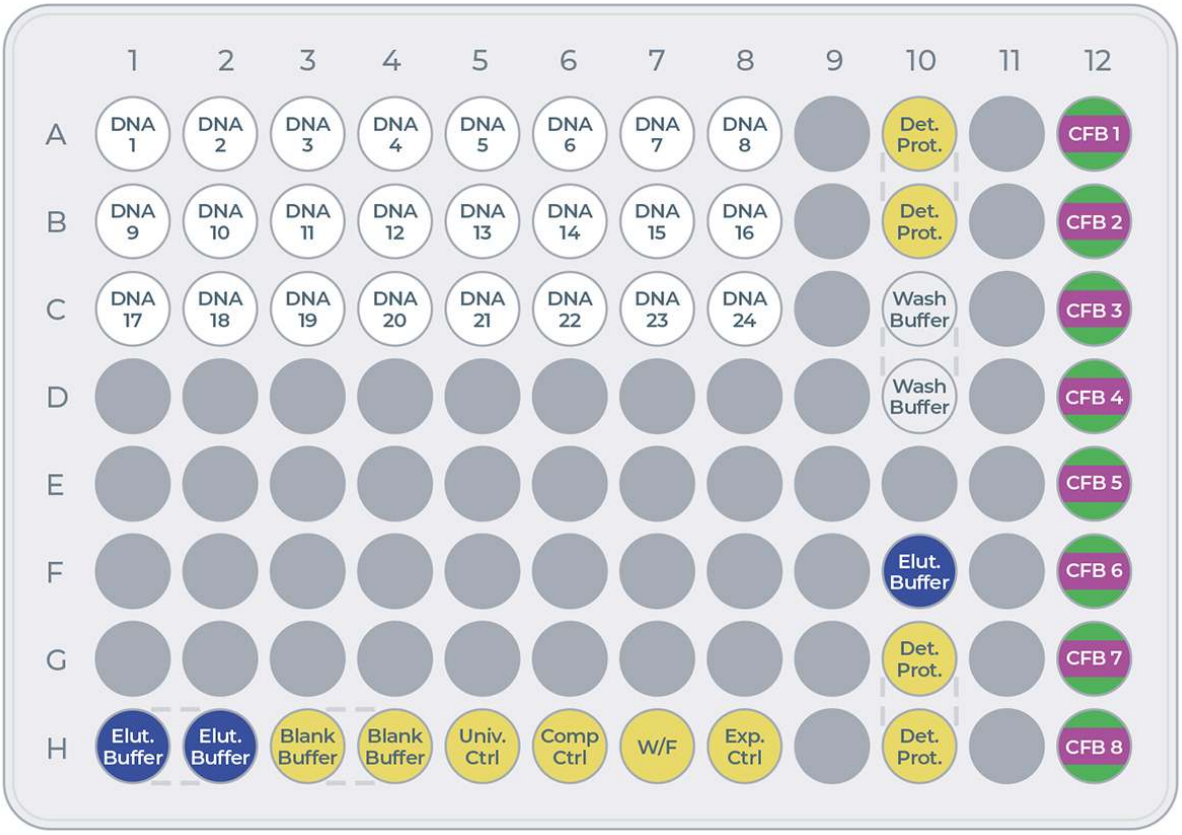



**Figure 5: Transfer plate layout**

| Reagent | Volume (µL) |
|---|---|
| eGene construct | 5 |

| Reagent | Volume (µL) |
|---|---|
| Controls: Blank Buffer, Universal Control (Univ. Ctrl), Complementation control (Comp. Ctrl), Full Workflow Control (W/F), Expression Control (Exp Ctrl) | 10 |
| Cell-free Blend (CFB): Cell-free Core Reagent + Additive 1 + Additive 2 | 20 (16+2+2) |
| Wash Buffer (Wash Buffer) | 16 |
| Elution Buffer (Elut. Buffer) | 10 µL in H1 & H2, 16 µL in F10 |
| Detector Protein (Det. Prot.) | 16 |

**Table 2: Reagents and volumes to load on the transfer plate**

**1.Take the Strep Beads from the fridge and the Cartridge Kit reagents (box with the yellow stripe on the label) from the -80°C freezer.**


**2.Place an empty 96-well transfer plate on ice.**

> **NOTE**
>
> The transfer plate should be kept on ice until the transfer of reagents to the Cartridge.
>
> Ensure you prepare the Cell-free Blends last.

**3. eGene constructs (DNA)**

Take the vials or the plates with the eGene constructs made in advance using the eGene Prep Kit out of the freezer and thaw on the benchtop at

room temperature. This takes approximately 15 minutes.
**Note:** the vials or the plates can be centrifuged for a few seconds to ensure all the liquid is at the bottom of the wells.

Load 5 µL of each eGene construct onto the transfer plate into wells: ▹ A1 to A8
▹ B1 to B8
▹ C1 to C8

**Note:** It is critical to load the eGene constructs onto the transfer plate in the exact order that they have been finalized in the experiment planned in the eProtein Discovery software.

**4. eProtein Discovery purification reagents** Thaw the Wash Buffer and the Elution Buffer on the benchtop at room temperature. Once thawed, vortex for 2 seconds and centrifuge for 2 seconds using a microcentrifuge to mix and collect all the reagents. ▹ Load 16 µL of Wash Buffer into wells C10 and D10.
▹ Load 16 µL of Elution Buffer into well F10
▹ Load 10 µL of ELution Buffer into wells H1 and H2

**5. eProtein Discovery controls** From the kit kept at -80°C, take the controls out and thaw them on ice.
▹ Load 10 µL of Blank Buffer into wells H3 and H4.
▹ Load 10 µL of Universal control into well H5.
▹ Load 10 µL of Complementation Control into well H6.
▹ Load 10 µL of Full W/F Control into well H7.
▹ Load 10 µL of Expression Control into well H8

**6. Strep Purification Beads** Strep Purification Beads are provided in 200 µL aliquots of 5% v/v suspension – To prepare the Strep Beads:

1. Take the vial of Strep Beads from the fridge and give it a quick spin for 2

seconds in a microcentrifuge to pellet the beads.

2. Resuspend the beads by gently pipetting up and down 10 times with a p200 pipette set on 90 µL.
3. Transfer 90µL of the resuspended beads into a 1.5 mL tube. Discard the rest only after the experiment starts, in case more volume is required.
4. Place the tube with Strep Beads on a magnetic particle separator and capture for 1 min.
5. Remove all the supernatant with a p200 pipette and discard the liquid.
6. Remove the tube with Strep Beads from the magnetic particle separator. Resuspend the beads in 100 µL Wash Buffer by slowly pipetting up and down 10 times.
7. Repeat steps 4 to 6 twice more for a total of three washes.
8. After the third wash, spin down the tube and place it back on a magnetic particle separator and capture for 1 min.
9. Remove all the supernatant with a p200 pipette and discard the liquid.
10. Spin down the tube, place it back on a magnetic particle separator and remove the residual buffer with a p20 pipette.
11. With a p20 pipette, resuspend the beads in 10.5 µL Wash Buffer by gently pipetting up and down 10 times to create a 15 µL 30% Strep Beads working
12. Keep the beads in the tube on the bench, not on ice.
    **Note:** The beads should NOT be loaded onto the transfer plate.

**7. Detector Protein** The Detector Protein is supplied as ready to use. Spin down the tube for 2 seconds to collect the full volume at the bottom. Load 16 µL of Detector Protein into wells A10, B10, G10, and H10 of the transfer plate.

**8. Preparation of the Cell-free Blends** For each expression screening experiment, up to eight 20 µL Cell-free Blends can be made by adding 16 µL of Cell-free Core Reagent, 2 µL of a first additive, and 2 µL of a second

additive.

**Note:** The total volume of blend should always be 20 µL final
**Note:** the same additive can be used as first and second additive, for example 2 x 2 µL of Additive Buffer. The list of Additives is in Table 1.

1. Thaw on ice Cell-free Core Reagents and Additives
2. Once thawed, vortex for 2 seconds the Cell-free Core reagents and Additives to ensure they are well mixed.
3. Centrifuge for 2 seconds the Cell-free Core reagents and Additives using a microcentrifuge to return any droplets to the bulk aliquot.
4. Add 16 µL of Cell-free Core reagent to wells A12-H12.
5. Add 2 µL of your first selected additive to wells A12-H12.
6. Add 2 µL of your second selected additive to wells A12-H12.

**Note:** It is critical to load the Cell-free Blends onto the transfer plate in the exact order that they have been finalized in the experiment planned in the eProtein Discovery software.

## Set up the experiment on the instrument

### Log in and select the experiment

1. Press the **[Power Switch]** to activate the Instrument power-up, initialization and self-test sequence.
2. Log into the instrument software by selecting the user and entering PIN (Figure 6a and 6b).

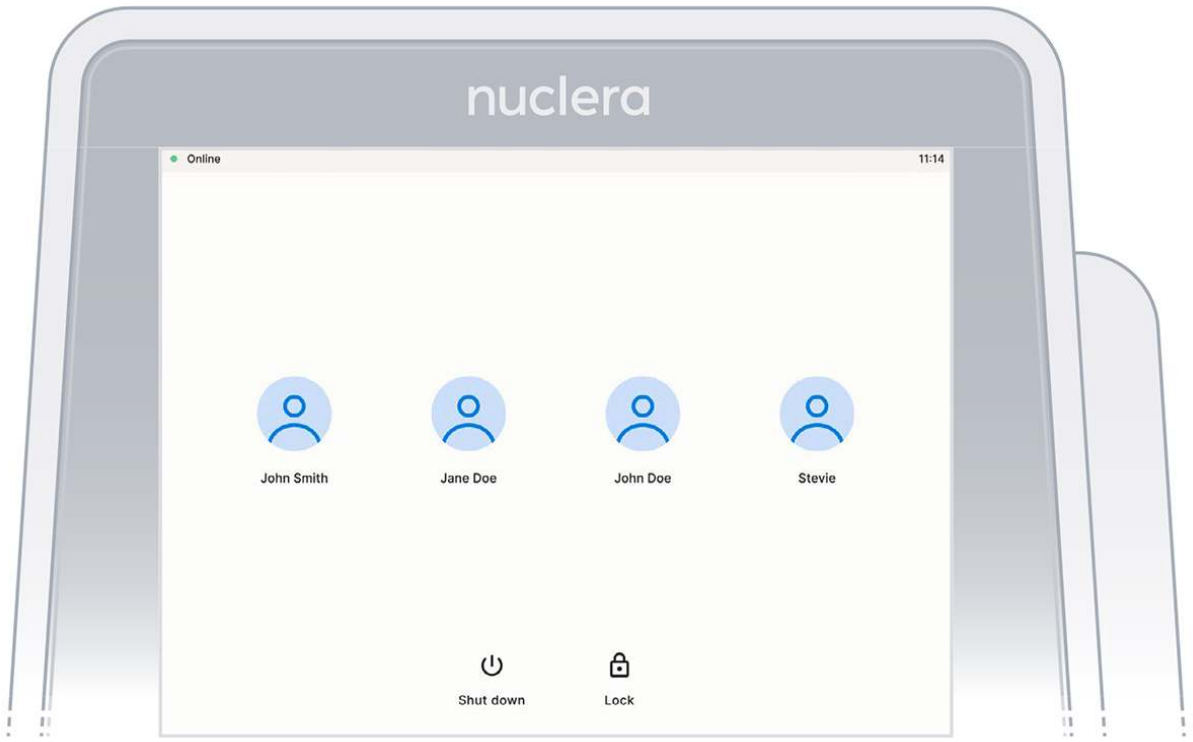



**Figure 6a: User accounts on the instrument software**

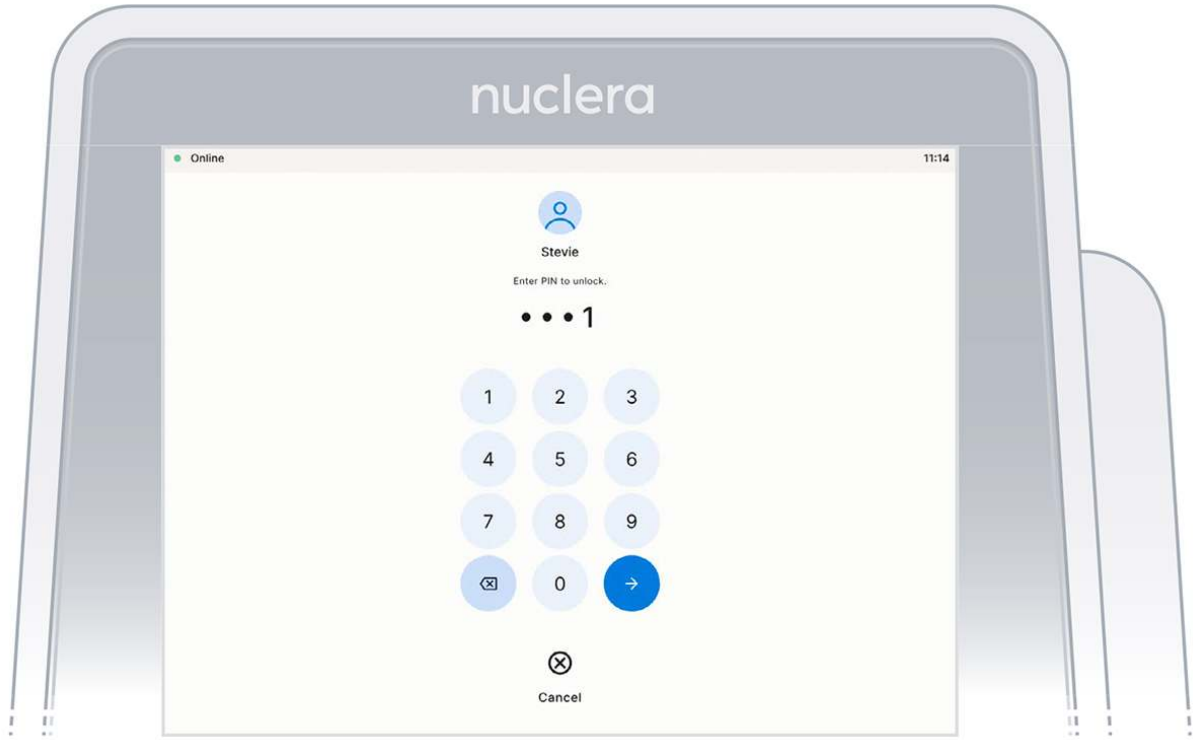


**Figure 6b: PIN Interface**

3. On the instrument software, select the experiment initially set up on eProtein Discovery software (Figure 7).

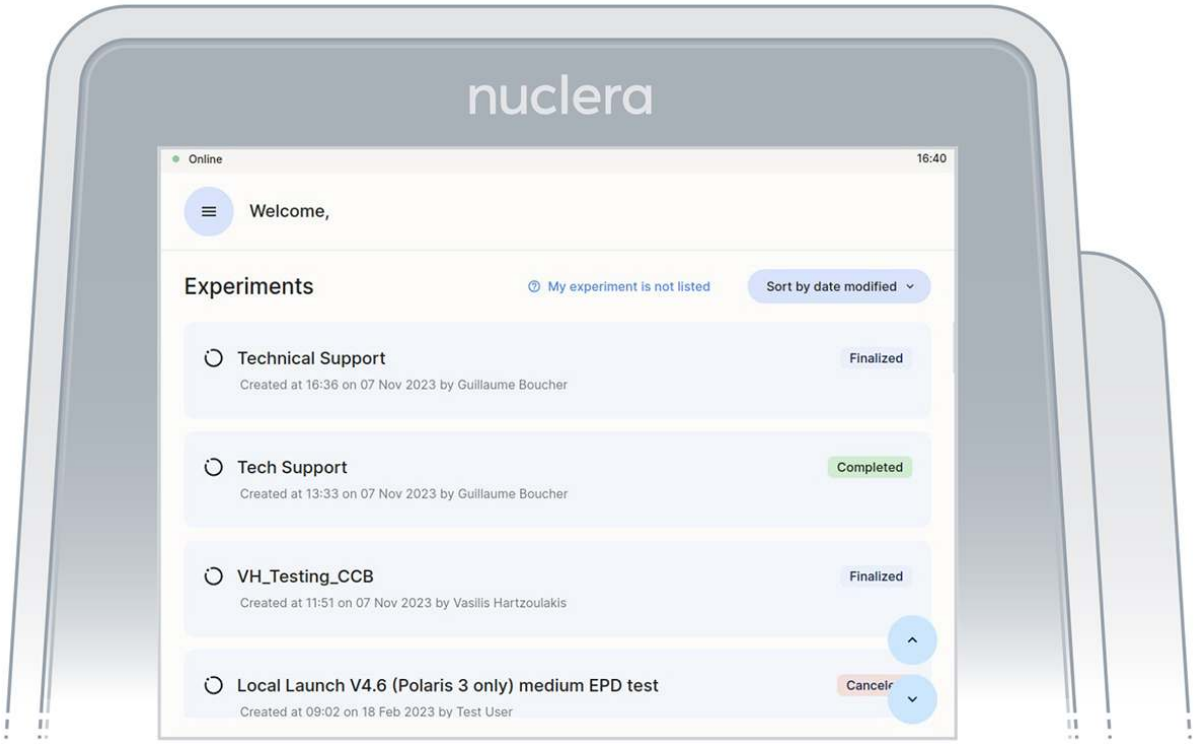



**Figure 7: Instrument software welcome page with the list of finalized experiments**

4. Read the Before you proceed section and press the **[Next]** button (Figure 8).

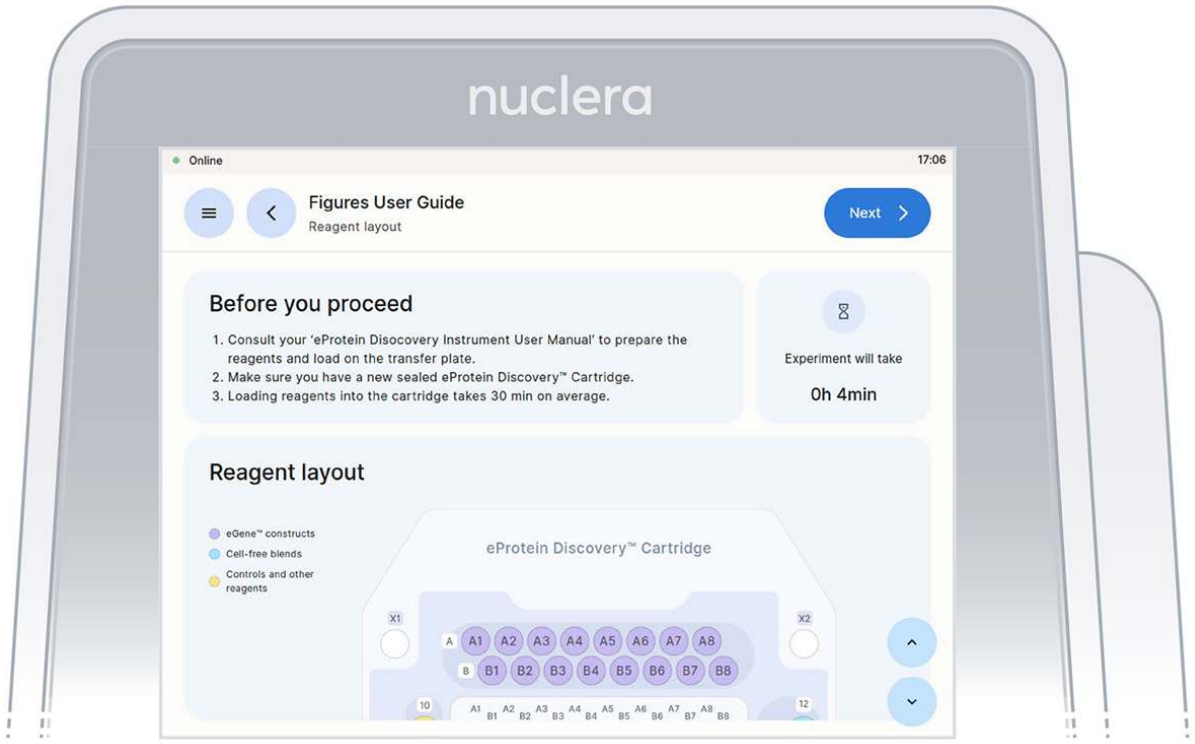



**Figure 8: Instrument software welcome page with the list of finalized experiments**

5. Get the transfer plate containing the reagents and cartridge ready.
6. Go through and tick the checklist, and press the **[Next]** button (Figure 9). The drawer will open.

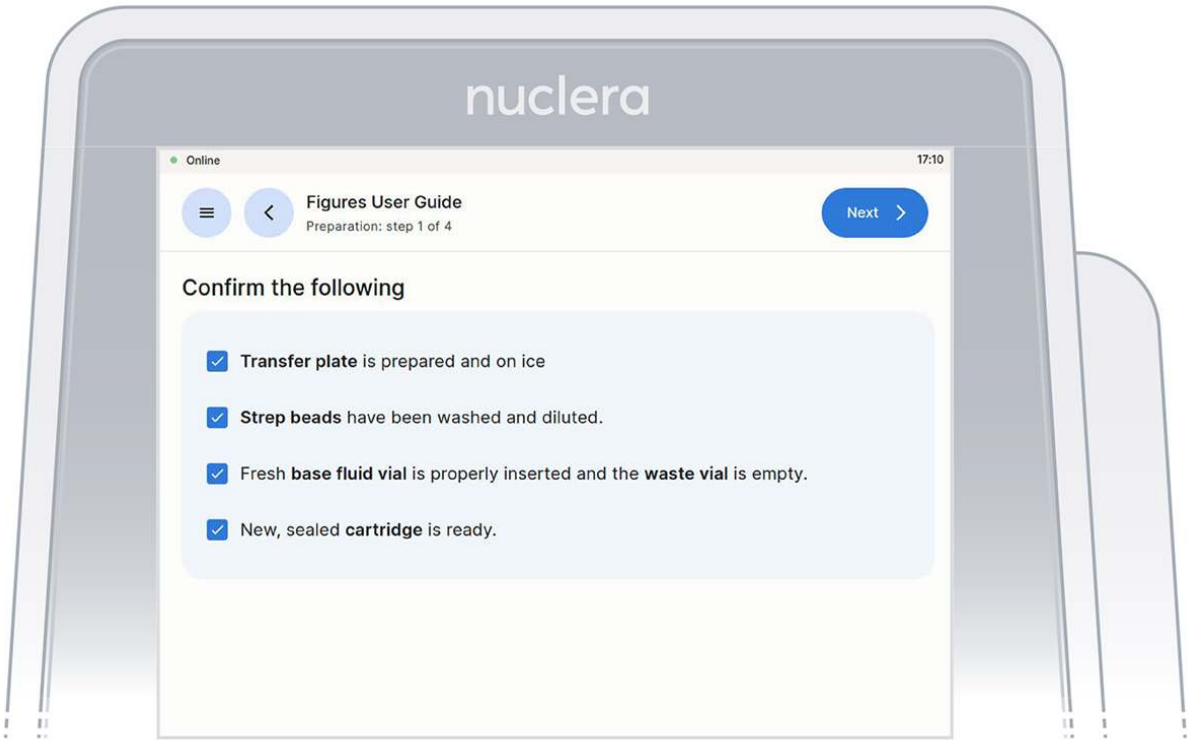



**Figure 9: Loading of the cartridge on the eProtein Discovery instrument.**

7. Unpack and load a cartridge as shown on the screen of the eProtein Discovery instrument, place the cover on the cartridge, avoid touching the electrical connectors, and press the **[Next]** button (Figure 10).


**Figure 10: Checklist screen before the experiment starts.**

**Note:** keep the cartridge packaging to dispose of the cartridge after use.

8. Keep cover on the cartridge. Markings on the cover will guide you through the loading process.

## Set up the pump on the instrument

**Follow the on-screen instructions to complete the experiment.**

▹ These instructions will guide you in operating the eProtein Discovery instrument and completing an experiment on the instrument.

▹ The instructions must be followed in the order shown on the screen.

▹ You can navigate forward and back through the steps using the buttons at the top.

▹ You can scroll up and down using the arrows at the bottom right of the screen when shown or with your fingers.

**Note:** once you start the experiment, the back button on the instrument will be disabled.

1. On the right hand side of the instrument, ensure the tubings for the integrated pump are placed in the tubing holder, and press the **[Next]** button (Figure 11).


**Figure 11: Verification screen that the tubings for the integrated pump are placed in the tubing holder.**

2. Ensure the vial of base fluid and the waste container have been connected to the pump located on the right hand side of the instrument. Press the **[Next]** button (Figures 12).

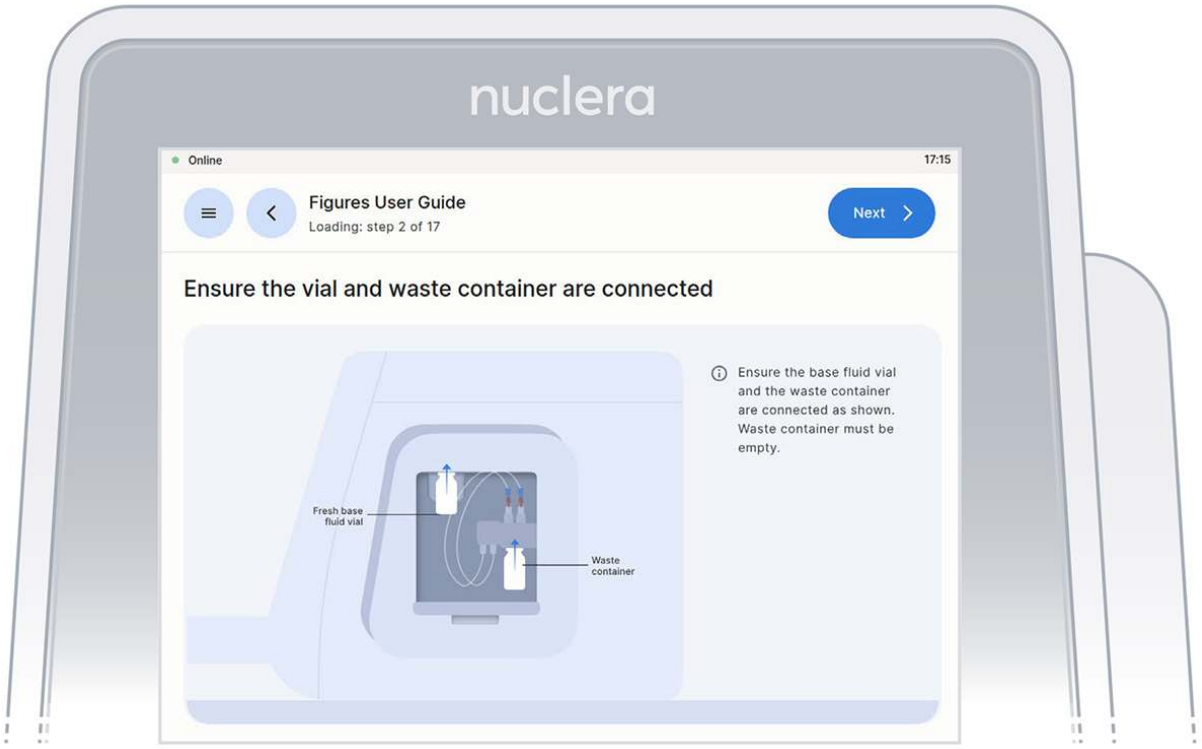



**Figure 12: Vial of base fluid and the waste container connected to the pump as shown on the screen**

## Filling the cartridge with base fluid

1. With the tubes and containers in place, ensure that some of the base fluid has dripped into the waste container (Figure 13).


**Figure 13: Priming the pump tubes with base fluid**

2. Remove the tube connectors from the holder, connect them tightly to the corner ports X2 and X3 of the cartridge, and press the **[Next]** button (Figure 14). Either connector can be interchangeably inserted into corner port X2 or X3.

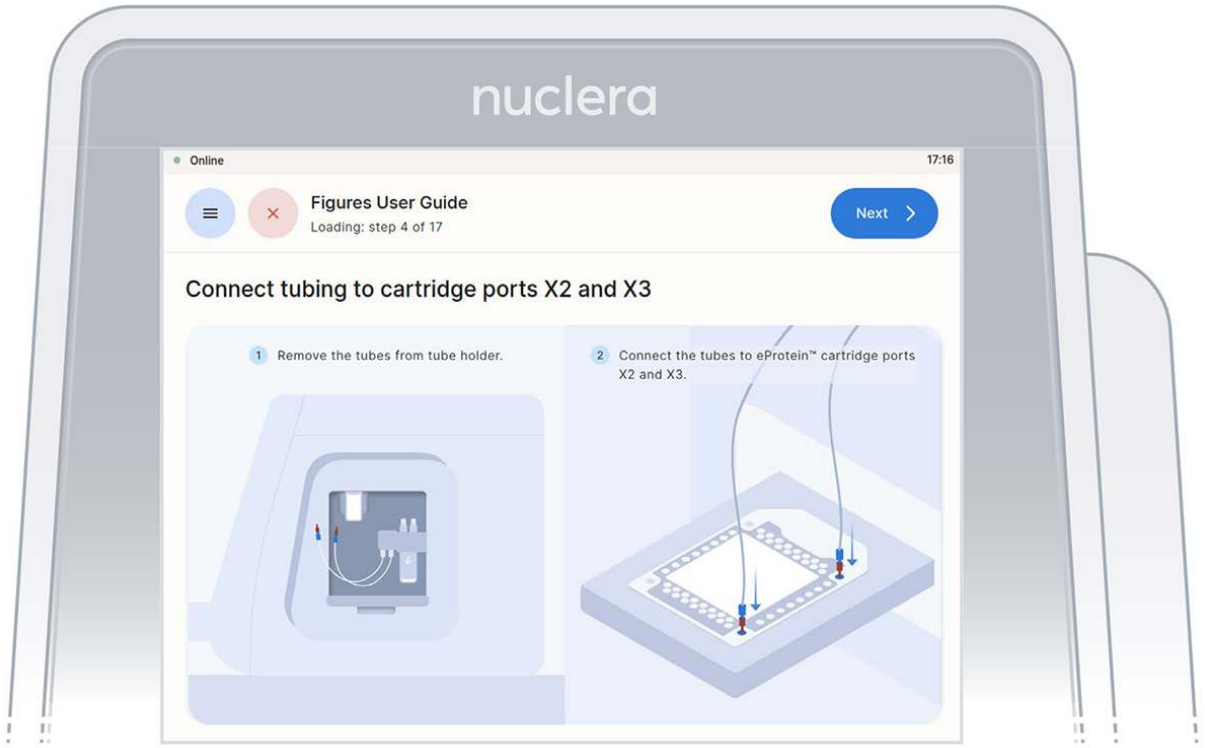



**Figure 14: Inspection that all the ports on the cartridge are filled with base fluid**

3. Inspect the cartridge for air bubbles that may have been introduced during the priming with base fluid.
   If any air bubbles persist after base fluid priming, use a single-channel p200 pipette to aspirate the air bubbles from the nearest port and reinject slowly the base fluid that was aspirated into a corner port (X1 or X4). Press the **[Next]** button (Figure 15).

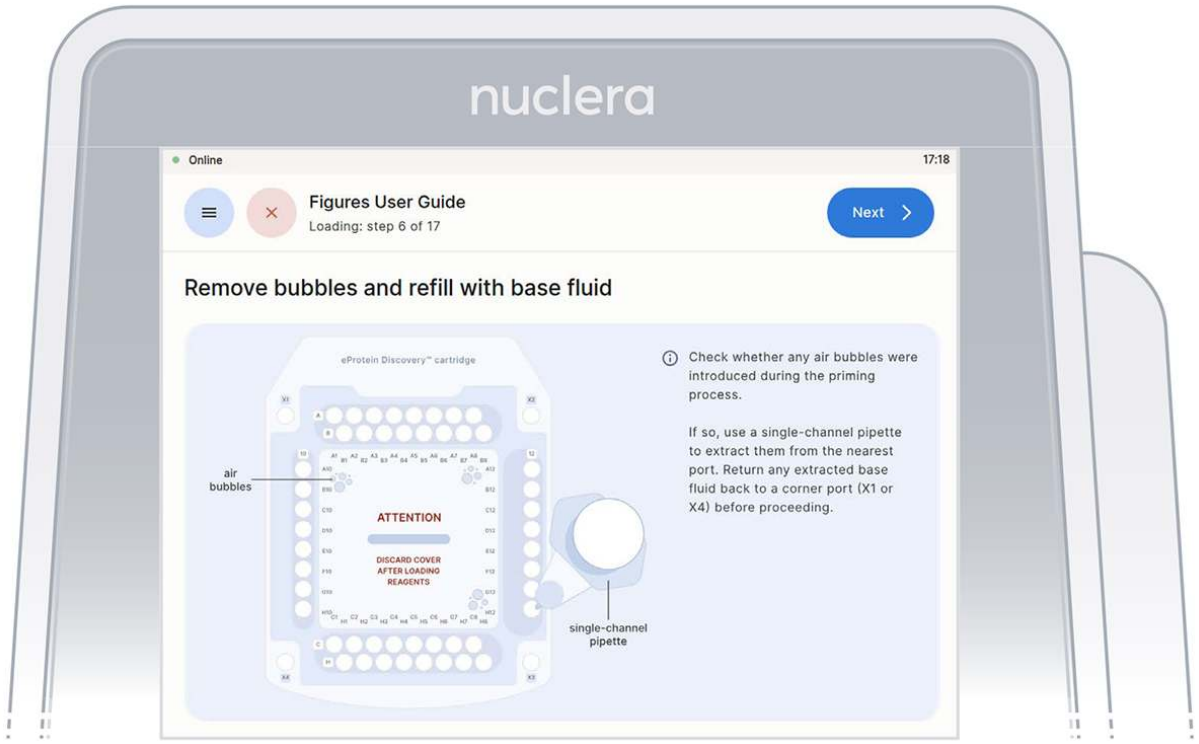



**Figure 15: Connection of the pump tubes to the cartridge**

4. Inspect the ports on the cartridge after the priming with base fluid is complete. Ensure all the ports are filled and press the **[Next]** button (Figure 16).

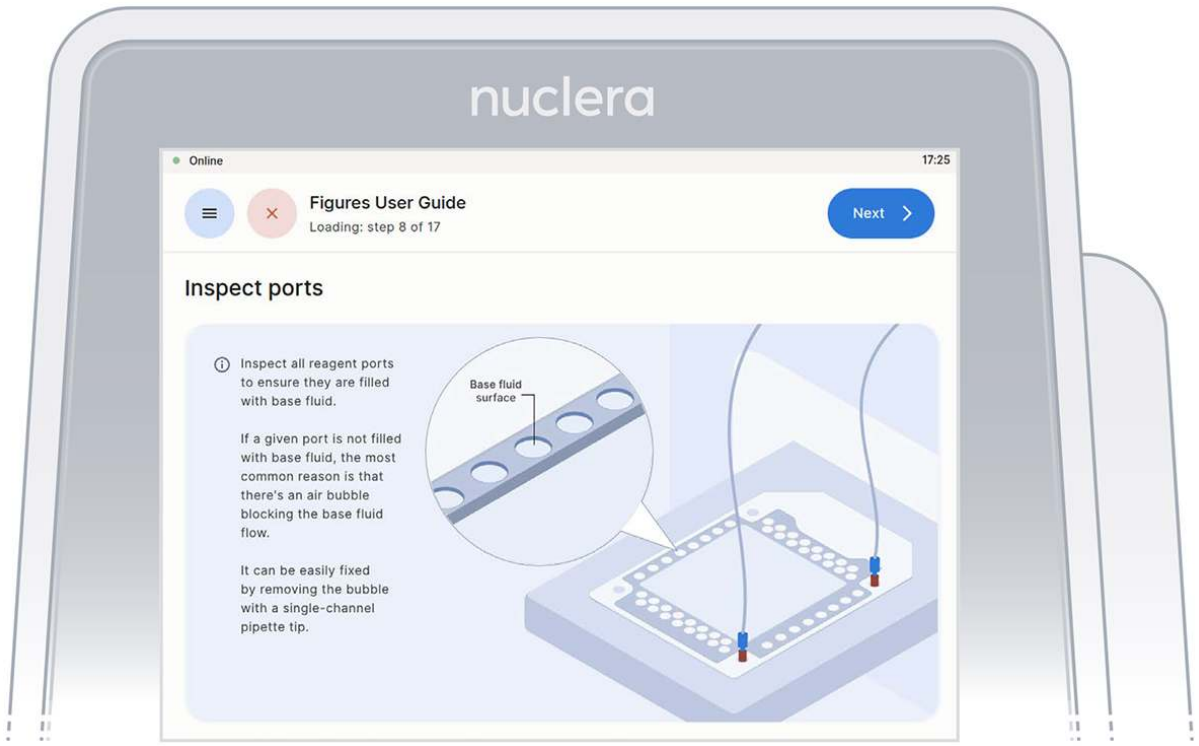



**Figure 16:Inspection that all the ports on the cartridge are filled with base fluid**

# Load the reagents on the cartridge

**Tips for a perfect loading:**

▹ Follow the on-screen instructions that will guide you in loading the reagents.

▹ The loading of the reagents should be done using an 8-channel pipette.

▹ To facilitate the pipetting of the reagents, the transfer plate can be moved from the ice bucket to the bench.

▹ Check the plate for the presence of air bubbles. Air bubbles can be removed by spinning the plate in a swing rotor centrifuge for about 10 seconds.

▹ After aspirating the reagents, make sure that all pipette tips are filled evenly, and contain no air bubbles.

▹ When loading the reagents into the ports, ensure the tips are immersed just below the surface of the base fluid, and dispense slowly until the first

stop of the pipette is reached.

▹ Do not pass the first stop as it would release air bubbles (Figure 17).

▹ Do not engage the pipette tips fully into the ports, the tip ends should not touch the bottom of the ports while dispensing the reagents (Figure 17).

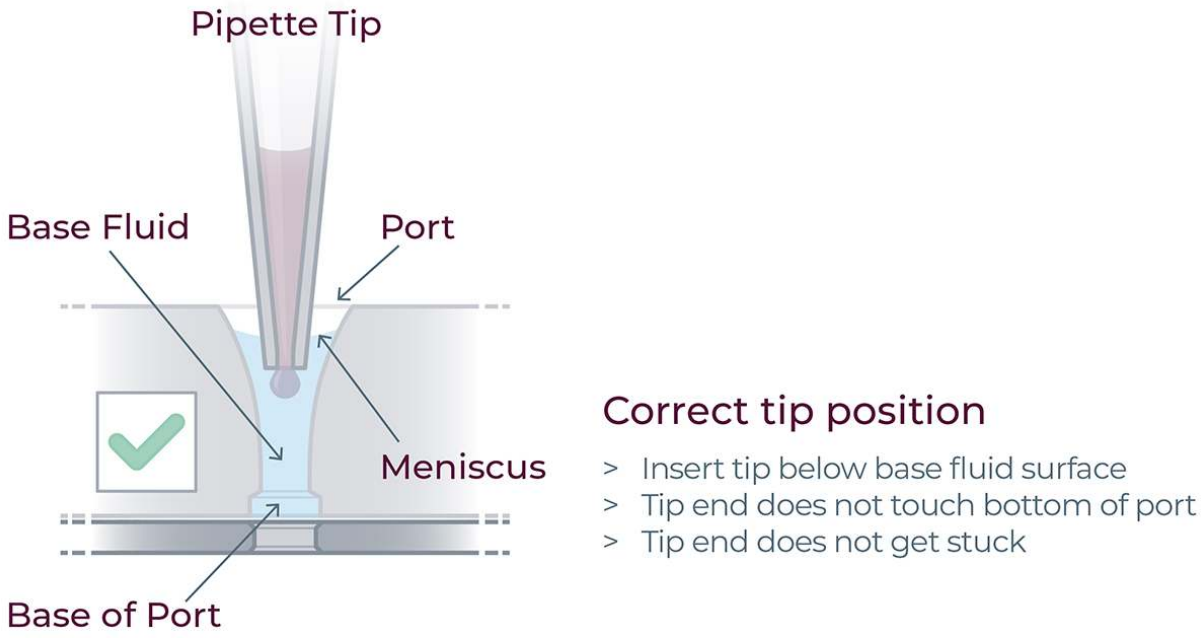



**Figure 17: For correct reagent loading the pipette tip is immersed in the base fluid and not touching the bottom of the port**

## Load eGene constructs - rows A, B and C

**IMPORTANT NOTE**

It is critical not to leave any port empty. If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit, **not with water.**

**Tip:** Empty ports can be used for duplicates.

▹ Load 8x fresh pipette tips and aspirate 3 µL of the eGene constructs from

the transfer plate wells A1-A8 into ports A1-A8 of the cartridge (Figure 18).
▹ Load 8x fresh pipette tips and aspirate 3 µL of the eGene constructs from the transfer plate wells B1-B8 into ports B1-B8 of the cartridge (Figure 18).
▹ Load 8x fresh pipette tips and aspirate 3 µL of the eGene constructs from the transfer plate wells C1-C8 into ports C1-C8 of the cartridge (Figure 18).
▹ Ensure the tip is immersed in the base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.
▹ Eject the pipette tips into a waste container.
▹ Press the [Next] button on the screen.

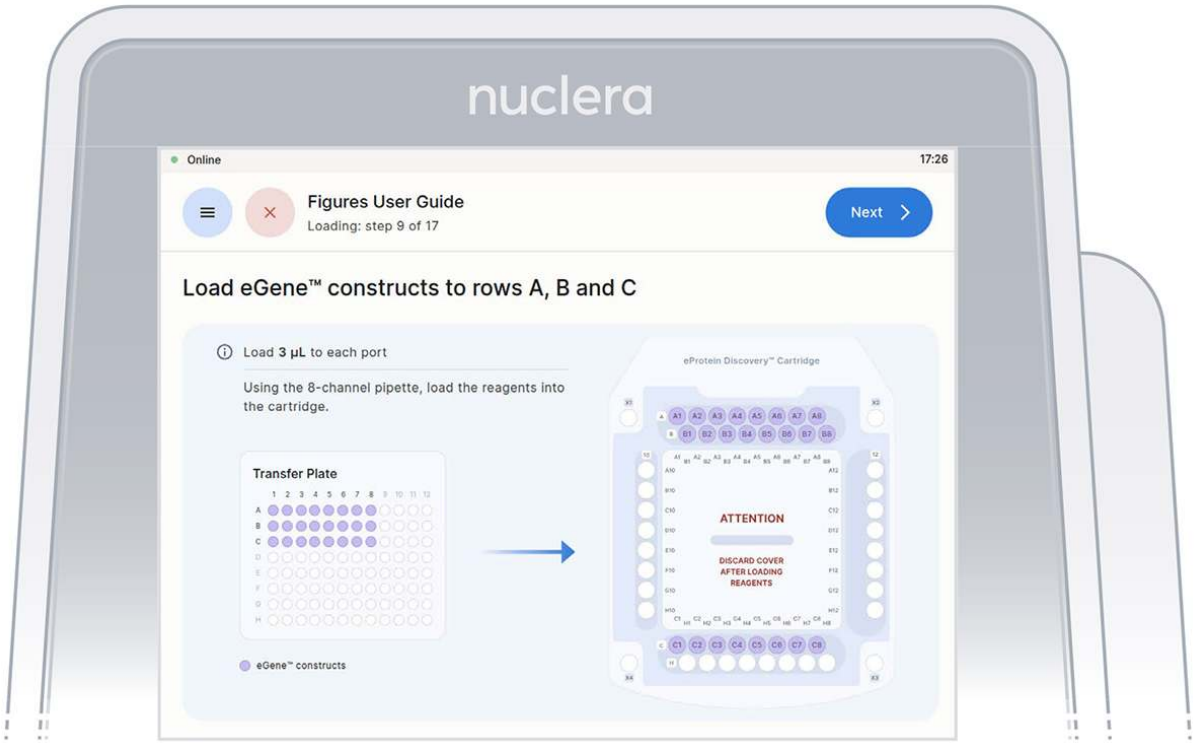



**Figure 18: Loading of the eGene constructs onto row A, B and C of the cartridge**

## Load reagents - row H, column 12 and column 10

**1. Reagents - row H:**

▹ Load 8x fresh p20 pipette tips and aspirate 3 µL of the reagents from the

transfer plate wells H1-H8 into ports H1-H8 of the cartridge (Figure 19).
▹ Ensure the tip is immersed in the base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.

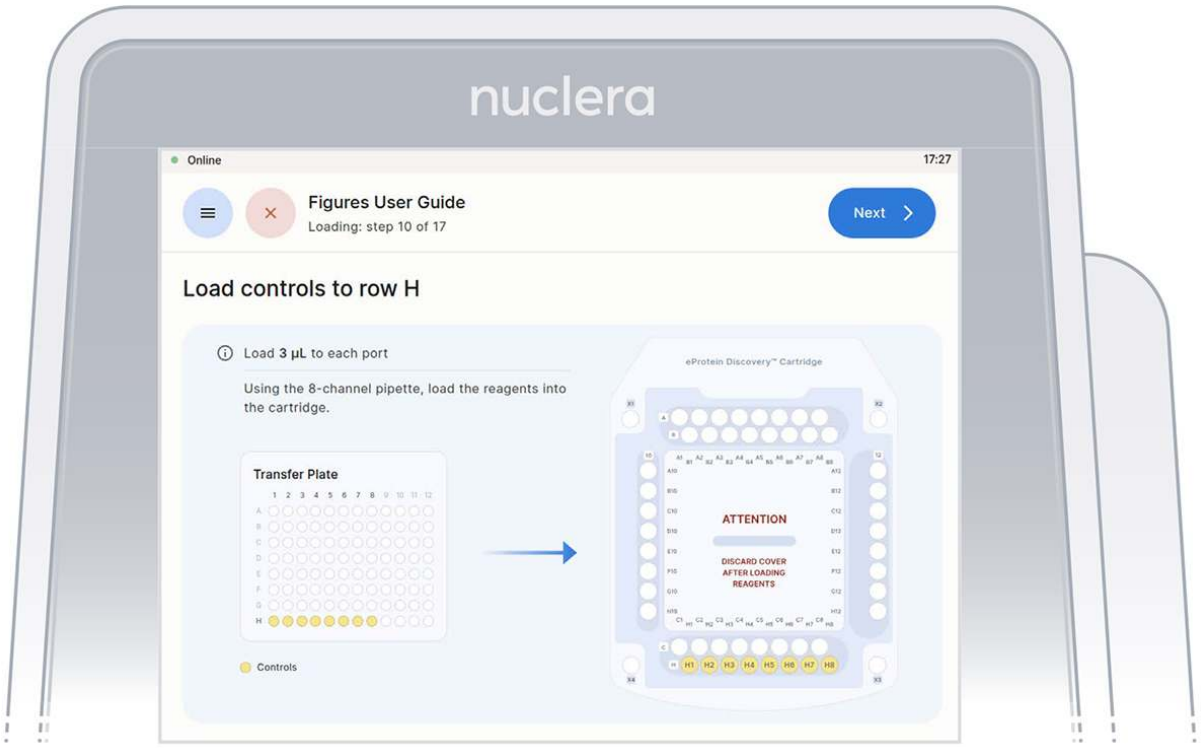



**Figure 19: Loading of the reagents onto row H of the cartridge**

### 2. Reagents - column 12:

▹ Load 8x fresh p20 pipette tips and **mix the Cell-free Blends in the transfer plate by gently pipetting up and down 12 times.**

> **IMPORTANT NOTE**
>
> Be careful not to introduce air bubbles in the ports.
> Aspirate 12 µL of the Cell-free Blends from the transfer plate wells

A12-H12 into ports A12-H12 of the cartridge (Figure 20).

▹ Ensure the tip is immersed in the base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.


**Figure 20: Loading of the Cell-free Blends onto column 12 of the cartridge**

### 3. Reagents - column 10:

▹ Load 7x fresh p20 pipette tips and aspirate 12 µL of the reagents from the transfer plate wells A10-D10 and F10-H10 into ports A10-D10 and F10-H10 of the cartridge (Figure 21).
▹ Ensure the tip is immersed in the base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.
▹ Eject the pipette tips into a waste container.

▹ Press the **[Next]** button on the screen.

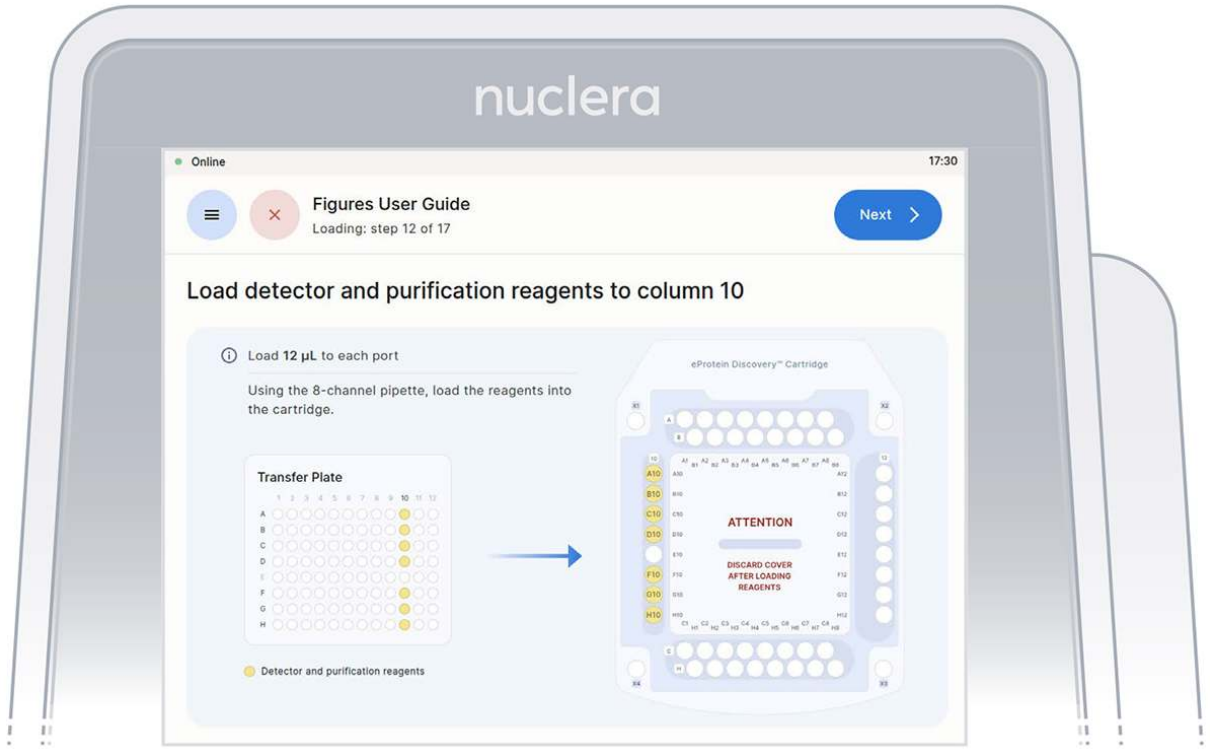



**Figure 21: Loading of the reagents onto column 10 of the cartridge**

### 4. Strep Purification Beads - port E10:

▹ Using a single channel p20 pipette, mix the Strep Purification Beads twelve times by gently pipetting up and down. Be careful not to introduce air bubbles. Aspirate 12 µL of the Strep Purification Beads prepared in a tube and dispense into port E10 of the cartridge (Figure 22).

▹ Ensure the tip is immersed in the base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tip fully into the port.

▹ Eject the pipette tip into a waste container.

▹ Press the **[Next]** button on the screen.


**Figure 22: Loading of the Strep beads to port E10 of the cartridge**

5. Remove the cover from the cartridge (Figure 23)

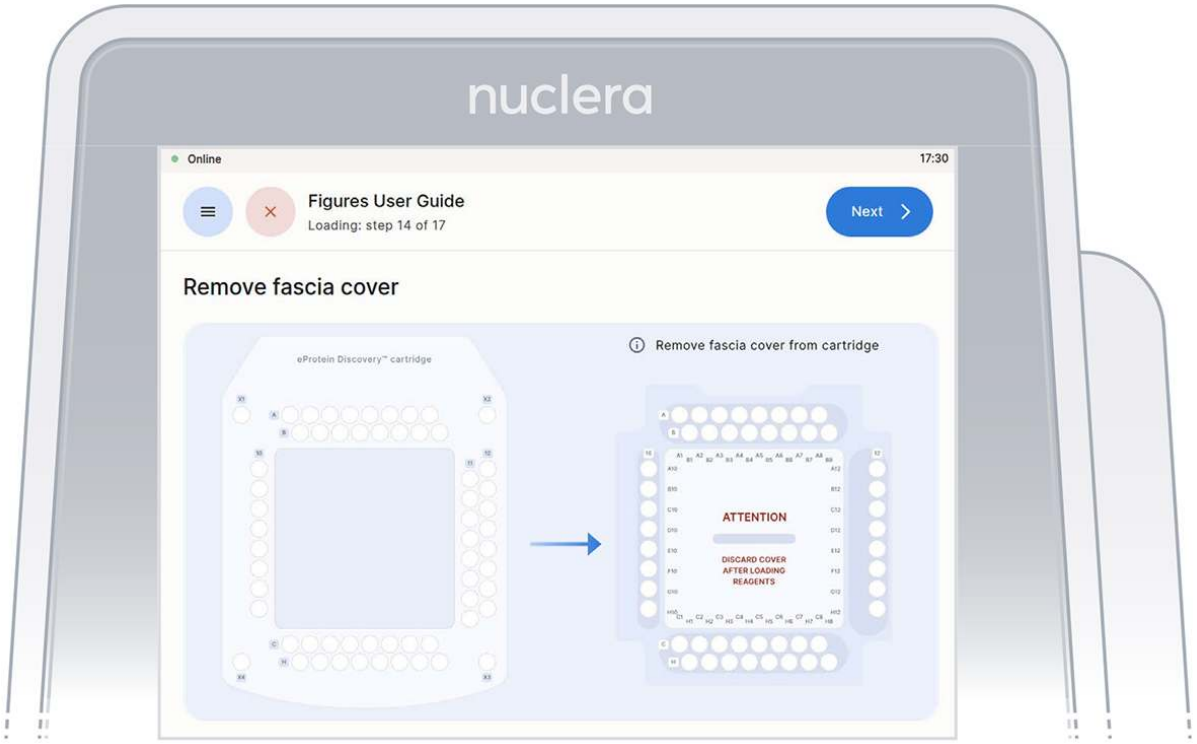



**Figure 23: Remove the cover from the cartridge**

# Load reagents in the cartridge

1. Press the **[Next]** button to start the aspiration of the base fluid and the loading of the reagents on the cartridge (Figure 24).

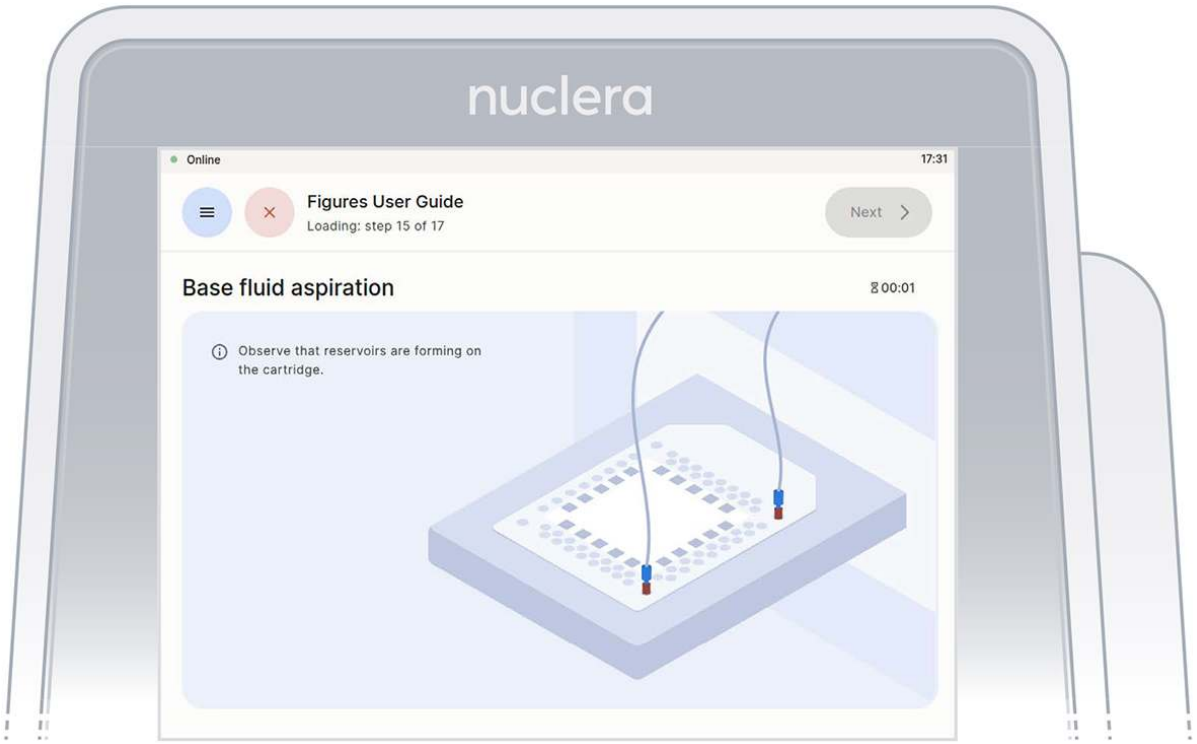



**Figure 24: Base fluid aspiration**

2. Disconnect the tubes from the cartridge and place them in the tube holder on the right hand side of the instrument. Press the **[Next]** button on the screen (Figure 25), and the drawer will close. Quality controls will be performed, and the drawer will open.

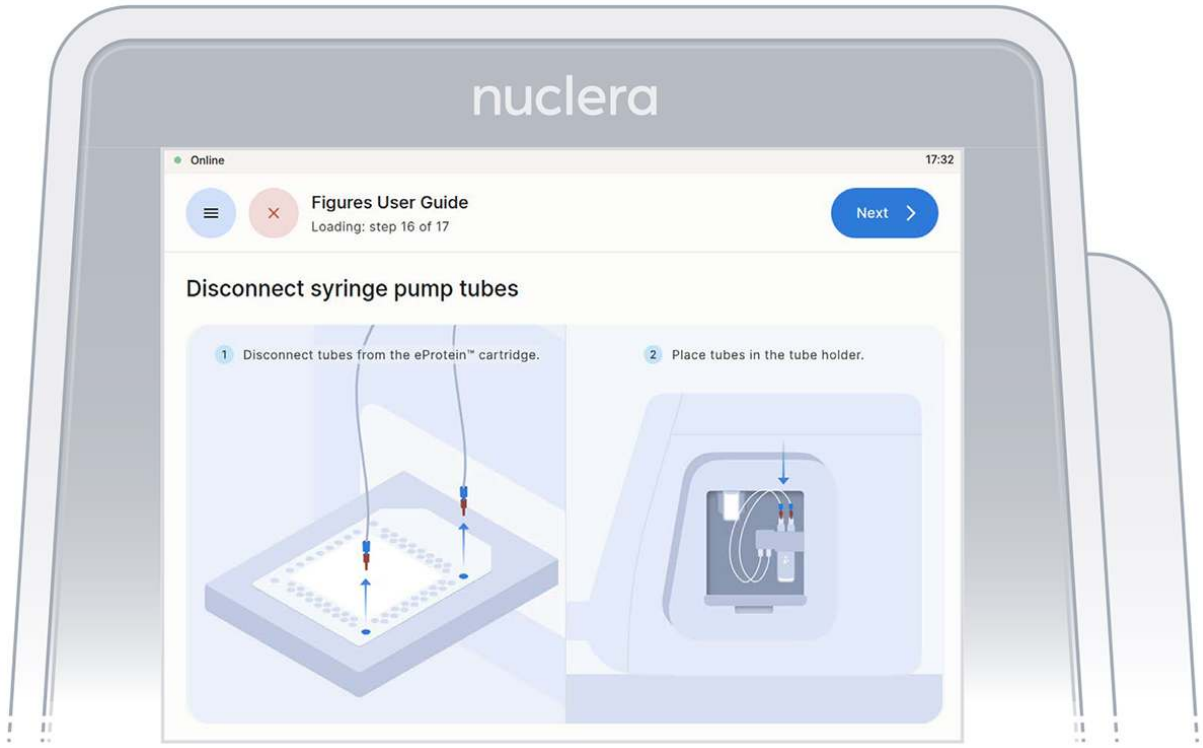



**Figure 25: Disconnect the tubes and place them on the tube holder**

3. Inspect the reservoirs have formed correctly in the cartridge as shown on the screen. If so, Press the **[Next]** button (Figure 26).

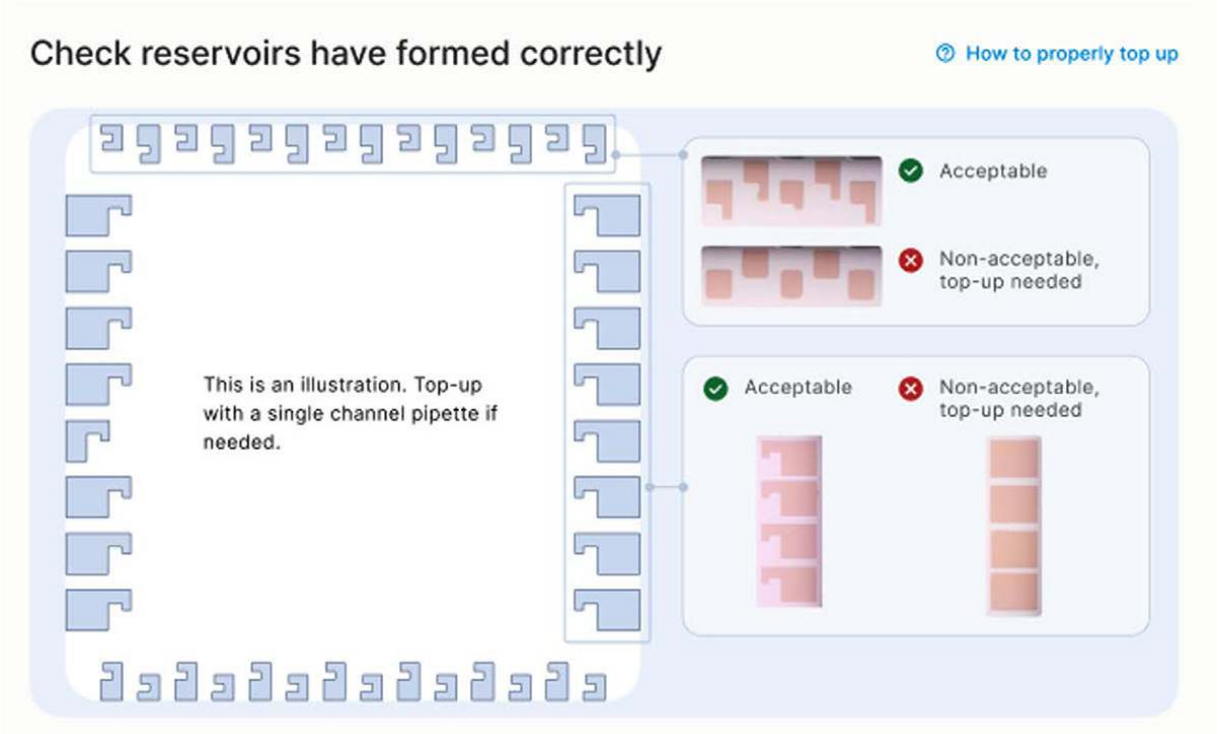


**Figure 26: Check the reservoirs have formed correctly on the cartridge**

**Note:** Any presence of a marker on the reservoir is acceptable, as shown on the top right of Figure 26

**Troubleshooting tip 1:**

If a reservoir is not properly formed, first fully engage a new empty pipette tip into the port and reach the bottom of the port. This action may trigger the correct formation of the reservoir.

**Troubleshooting tip 2:**

▹ If the shape of a reservoir is still not correct, remove the empty tip from the port, replace with a new tip, then add a small volume of the corresponding reagent using a p20 pipette with a pipette tip. Do not depress the pipette past the first stop as this could introduce air bubbles inside the cartridge.

▹ Re-engage the tip until reaching the bottom to the port and dispense the reagent slowly until correction is complete (Figure 27).

▹ The recommended volumes for manual correction are:

▹ 1.5 µL for ports in rows A, B, C or H

▹ 3 µL for ports in columns 10 or 12

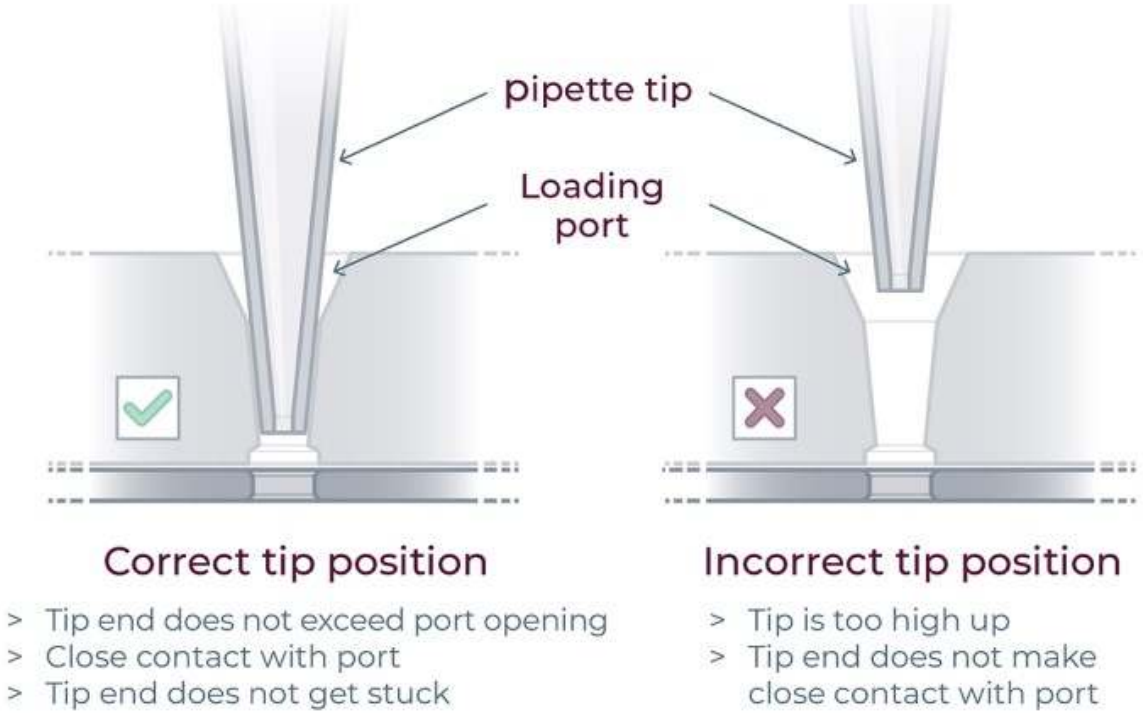



**Figure 27: For manual correction of the reagent loading to correctly form the reservoirs on cartridge**

# Analyze the results

### Instrument software results screen

After completion of the experiment, the results are shown on the instrument screen. The four best obtainable combinations of eGene and Cell-free Blend are displayed with the predicted in-tube scale-up yields (Figure 28). Further analysis can be carried out from the eProtein Discovery Cloud Software.

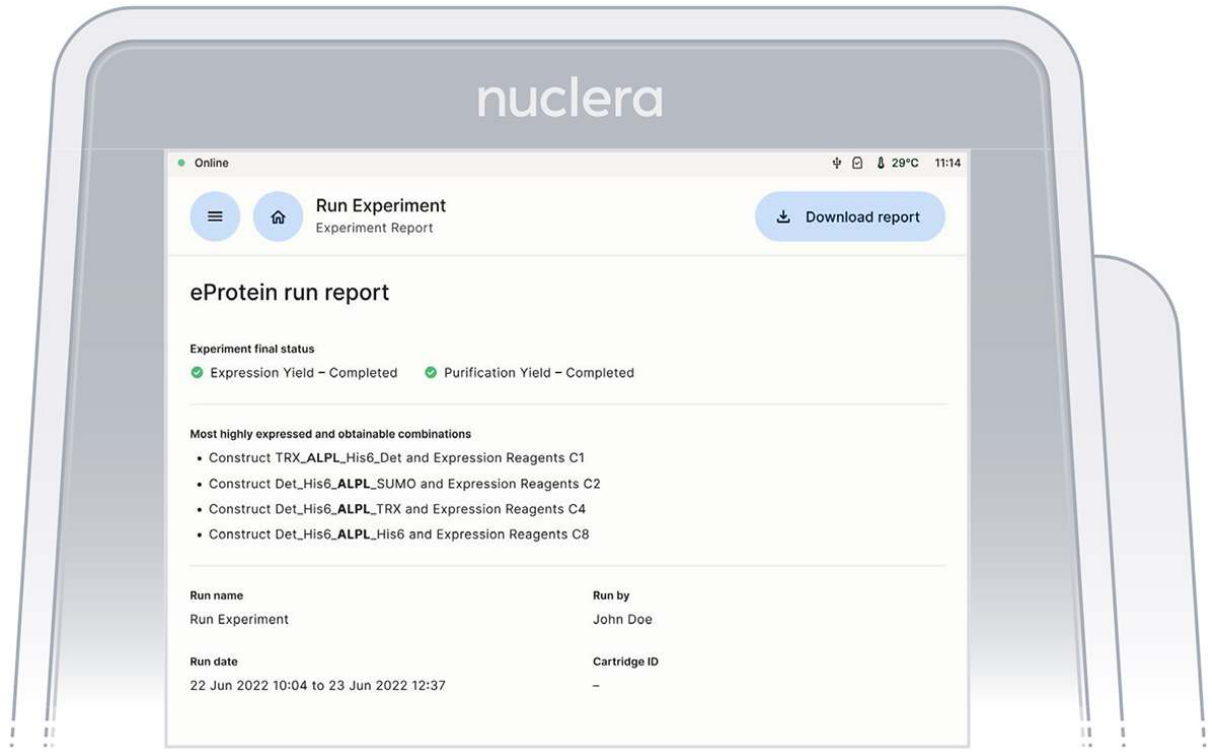



**Figure 28: Result screen from the Instrument Software**

## eProtein Discovery Cloud Software report

At the end of the experiment a report containing all the information about the experimental setup is transferred onto the eProtein Discovery Cloud Software. The upload takes about 15 minutes and during this time the **[Download Report]** button at the top right corner of the screen is grayed out.

**Note:** the instrument should not be switched off until the report is transferred and becomes available on the eProtein Discovery Cloud Software.

**The experiment report contains:**

▹ **Experiment video**

The video should be watched to ensure the correct operation of the instrument and cartridge during the experiment. Any questions or concerns regarding the operation of the droplets should be directed to the

Nuclera Technical Support team (techsupport@nuclera.com).

▹ **PDF report file**

The PDF report file is a summary of the experiment setup and the results, saved in the report folder with the name given to the experiment included in the file name

▹ **CSV report file** The report file is a csv file saved in the report folder with the name given to the experiment included in the file name. The results for each one of the 30 purified target protein conditions, and the 192 conditions for the produced protein are listed in the csv file. It also contains the measured values for the controls, the expected range for the controls, and a PASS/FAIL score if the measured values are within the expected range.

▹ **Blue light images (TIFF images)**

Images acquired at the end of expressions and purification. These images can give the user information about the solubility of the protein.

▹ **Other files**

The folder contains additional files that can be used by the Nuclera Technical Support team for troubleshooting purposes

## Finishing the experiment

1. Remove the cartridge from the instrument drawer by lifting it as shown on the screen and place it in its original packaging. Press the **[Next]** button (Figure 29).

**Note:** there is no need to drain the base fluid out of the cartridge.


**Figure 29: Remove the cartridge from the instrument**

2. Remove the waste container from the holder, empty its content, and place it back on the instrument.
3. Remove the vial of base fluid and dispose of it with biohazard sharps waste container according to local waste disposal rules and regulations.

**Note:** Do not reuse consumed cartridges and dispose of any residual reagents, kits are intended as single use only.

4. Dispose the packaged used cartridge in a biohazard sharps container, according to local waste disposal rules and regulations.
5. The experiment report is available for download from the eProtein Discovery software.
6. Power down the instrument after use by pressing the [Power off] button (Figure 30)

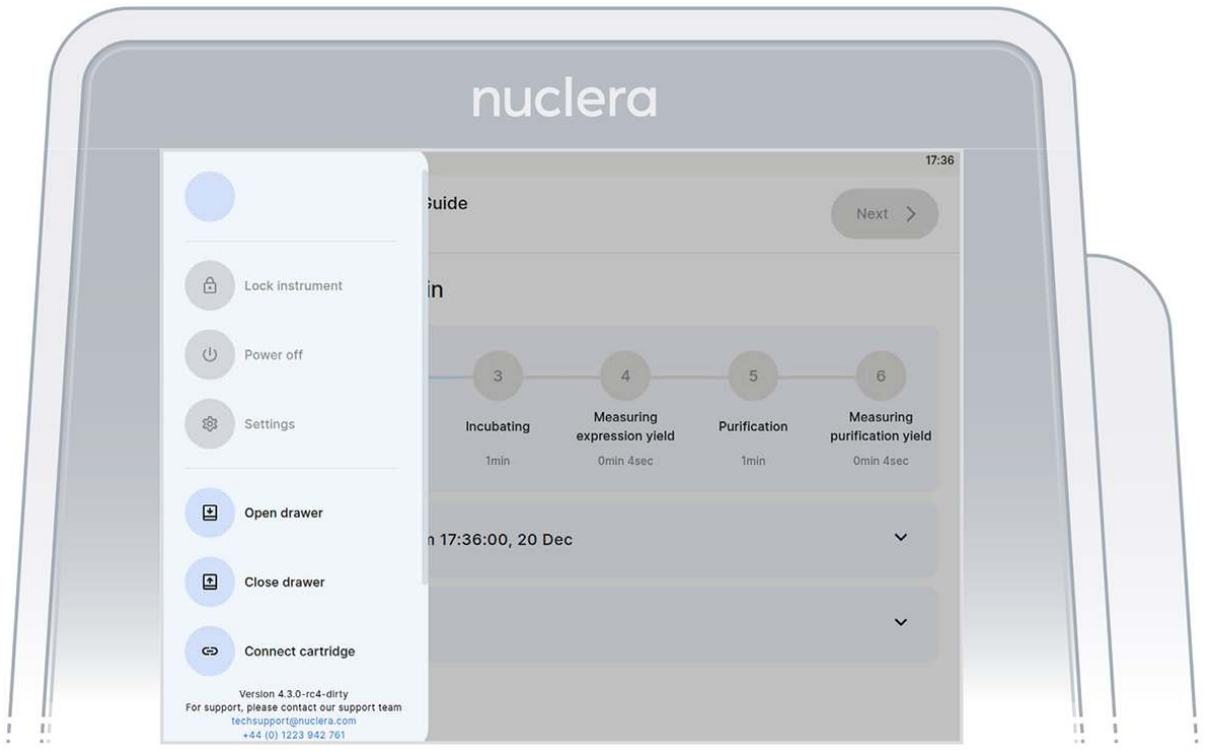



**Figure 30: Remove the cartridge from the instrument**

# Frequently Asked Questions (FAQ)

## 1. General

| Questions/ Issues | Answers |
|---|---|
| Where can I suggest future improvements? | Please email us at techsupport@nuclera.com, your feedback is very important to us as it allows us to improve the instrument, the technology and our services. |
| A component or reagent is missing | If a component or a reagent is missing, please contact the Nuclera Technical Support team |

## 2.Technical issues (software / hardware)

| Questions/ Issues | Answers |
|---|---|
| The eProtein instrument doesn't start (blank screen and no light) - no power | 1. Check it's plugged in<br>2. Check the socket is working.<br>3. Please inform the Nuclera Technical Support team. |
| WiFi is not connected | 1. Check that there is internet connection through the ethernet cable using a computer<br>2. Check that the instrument is connected to the internet through an ethernet cable.<br>3. If no experiment is running and the data export has completed, restart the instrument. |
| On the eProtein Discovery software, I don't remember my username / password | Search for the email received from "eProtein Cloud Software". You can also contact your administrator or the Nuclera Technical Support team to reset your password. |
| On the | Contact your administrator or the Nuclera Technical |

| Questions/ Issues | Answers |
| --- | --- |
| Instrument Software, I don't remember my username / password | Support team |
| On the eProtein Discovery software, my username and password are correct but not recognised | Contact your administrator or the Nuclera Technical Support team. |
| On the Instrument Software, my username and password are correct but not recognised | Contact your administrator or the Nuclera Technical Support team. |

| Questions/ Issues | Answers |
| --- | --- |
| The screen of the eProtein Discovery instrument is on but not responding | During the experiment it is possible that the touch-screen will stop responding. If this happens, the software is still running in the background. It is possible to remotely access the instrument desktop from another computer. Please inform the Nuclera Technical Support team. |
| One of the reservoirs is not formed as shown on the instrument | For manually topping up reservoirs, draw 1.5 µL to 3 µL of each desired reagent into a single channel pipette and load with gentle pressure in each port, holding the plunger for about 30 sec to 1 min. Once the fluid connection from the port is made to the existing liquid, wait until the reservoir is fully formed and gently remove the pipette tip from the hole. |
| It looks like my reagents merged together on the cartridge. | Depending on which reagents, it is likely that the results of the experiment will be compromised. It is up to the user whether they wish to continue the experiment at this point or to abort the run. Please inform the Nuclera Technical Support team. |
| Reagent port not filled with base fluid | If a given port is not filled with base fluid, the most common reason is that there is an air bubble blocking the base fluid flow. This can be |
| easily fixed | |

| Questions/ Issues | Answers |
| --- | --- |
| by removing the bubble with a single-channel pipette tip | |

**Nuclera Technical Support:**
UK Phone: +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

# eProtein Discovery™ System - Cloud-Enabled - Membrane Protein Workflow

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## General information

eProtein Discovery™ is the only end-to-end protein screening system that accelerates construct design, expression, solubility characterization and purification of target proteins. Accelerating your journey to soluble, purified protein.

- Rapid protein screening enables progress by allowing scientists to gain awareness quickly about which proteins – and which variations of a protein – will work.
- Simultaneously screen multiple constructs and protein expression conditions for soluble expression, and then scale up to micrograms of recombinant protein off cartridge to test in your applications.
- Explore diverse DNA constructs and screen various protein mimetics to identify the optimal combination for producing purified, stable membrane proteins..

  **Instrument:** With the eProtein Discovery™ System, you can draw a

straight line from theory to reality, allowing you to test hypotheses more efficiently and focus on promising targets. The eProtein Discovery™ instrument puts rapid protein screening on your benchtop. Designed for all levels of scientist, it streamlines your workflow and grants you the ability to identify optimal DNA constructs, test expression feasibility earlier, and pursue targets with confidence. Fail fast, succeed faster!

**Software:** eProtein Discovery™ software simplifies a complex multivariate experimental design. The software sets up and simultaneously tracks 88 different combinations of DNA sequences, flank pairs and expression reagent reactions performed on eProtein Discovery™ system. AI performs highly rigorous QA checks during an experiment to ensure data quality and consistency. Informative reports are then generated and exported that you can share with your team and beyond.

**Cartridge:** Powered by digital microfluidic technology, software controlled digital signals guide the movement of droplets on the eProtein Discovery™ Cartridge surface to enable splitting, dispensing and merging of biological reagents. Pipette DNA, cell free expression reagents and purification solutions on the Cartridge and the technology will automate the rest. Gain precise control of your eGene™ constructs and reagents to screen and discover optimal expressing conditions within 24 hours, accelerating target selection. A simple set-up allows anyone to run the system with minimal training.

**Reagents:** The reagents within the eProtein Discovery™ system allow you to optimize protein obtainability by characterizing and purifying different combinations of DNA constructs and expression conditions. Our system will screen and purify 88 different combinations in 22 hours for you to select the optimal conditions to scale up and get protein.

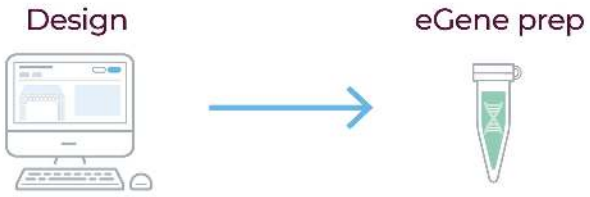
Our eProtein Discovery™ software will guide you in creating the panel of

DNA constructs and reagents to power your experiment. Our complete reagent package includes design and ordering of DNA, simplifying your workflow.

# eProtein Discovery Membrane Protein workflow

## Step 1: Design & Prep

Design, order and prepare linear DNA expression constructs

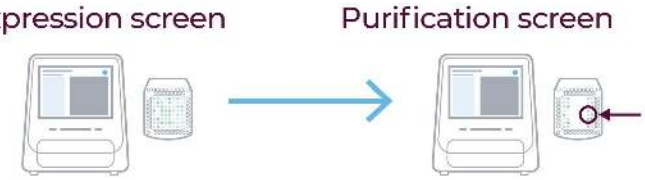



Input protein sequences into software and customize construct (eGene™) design with pre-set fusion tags

Prepare expression constructs with the included eGene™ Prep Kit

## Step 2: Load & Screen

Pipette and forget! Automated screens to determine your path to obtainable, purifiable proteins




Rapidly screen 88 expression conditions assessing attainable yield

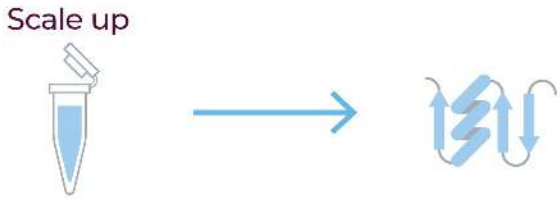
11 eGenes × 8 Customizable Cell-Free Blends = 88 Expression datapoints

Data driven selection of 88 expression conditions for a strep purifiability assessment to determine purifiable yield

88 Purifiability data points

## Step 3: Scale up & Go!

Scale up proteins at µg- and mg-scale




Choose winning eGene/Cell-Free Blend combinations based on screen datapoints for scale up and purification

µL scale included and mL-scale available

µg to mg purified protein (strep)

**Total time: < 48 hours to µg of protein**

(Hands on time < 2 hours)

# eProtein Discovery Bundle

### Equipment

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| eProtein Discovery Instrument | 1 unit | Room Temperature | N1001 |  |

---

### Cartridge Kit NC3006 - Consumables

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| eProtein Discovery Cartridge | 1 unit | Room Temperature | NC3006 |  |
| Base Fluid | 1 unit | Room Temperature | NC3007 |   |

---

**Cartridge Reagent Kit: Membrane Protein -80°C reagents - NC3013-1 (purple stripe on label)**

| Description | Quantity | Storage Temperature | Tube Reference ID | |
|---|---|---|---|---|
| Cell-Free Core Reagent | 160 µL | -80°C | SC3-01 |  |
| Blank Buffer | 150 µL | -80°C | SC3-02 | |
| Detector Protein* | 75 µL | -80°C | SC3-03 | |
| Universal Control* | 20 µL | -80°C | SC3-04 | |
| Expression Control* | 20 µL | -80°C | SC3-06 | |
| Wash Buffer* | 3 mL | -80°C | SU2-02 | |
| Elution Buffer* | 300 µL | -80°C | Su2-03 | |
| Additive Buffer* | 50 µL | -80°C | SC3-10 | |
| PDI/GSSG | 50 µL | -80°C | SC3-11 | |

| Description | Quantity | Storage Temperature | Tube Reference ID | |
|---|---|---|---|---|
| Mix* | | | | |
| TRxB1* | 50 µL | -80°C | SC3-12 | |
| DnaK Mix* | 50 µL | -80°C | SC3-13 | |
| Zinc Chloride | 50 µL | -80°C | SC3-14 | |
| Calcium Chloride | 50 µL | -80°C | SC3-15 | |
| Manganese Chloride | 50 µL | -80°C | SC3-16 | |
| Cofactor Mix* | 50 µL | -80°C | SC3-17 | |
| GSSG* | 50 µL | -80°C | SC3-18 | |
| 3C protease* | 50 µL | -80°C | SC3-19 | |

Reagents must be used before the expiration date indicated on the kit box.

* Single use reagent that cannot be freeze/thawed multiple times.

### Cartridge Reagent Kit: Membrane Protein +4°C reagent - NC3013-2

| Description | Quantity | Storage Temperature | Tube Reference ID | |
|---|---|---|---|---|
| Strep Beads | 2x 200 µL | +4°C | NC3010-2 |  |

### User supplied reagents

- 5 nM eGene constructs (DNA), or 100 nM Circular eGene stored at -80°C, generated using the Nuclera eGene Prep kit NC3008 or NC3009

### User supplied equipment

- Magnetic particle separator (compatible with 1.5 mL microcentrifuge tubes)
- Vortexer
- Microcentrifuge
- 1.5 mL microcentrifuge tubes
- 2-20 µL 8-channel pipette
- 2-20 µL single-channel pipette
- 200 µL compatible tips

**IMPORTANT NOTE**

We recommend using a manual multichannel pipette. However, if you do not have a manual multichannel pipette and/or prefer to use an electronic pipette, the electronic pipette must be configured correctly for use with the eProtein Discovery System. Improper use of electronic pipette can result in the introduction of air bubbles during loading and can lead to a run failure. If using electronic pipette, the following settings must be enabled:

1. Disable blowout/purge function
2. Avoid high speed aspiration
3. Avoid high speed dispensing
   Please contact Technical Support (techsupport@nuclera.com) for any questions. Any run errors resulting from improper use of electronic pipettes are the responsibility of the user.

## Protein Variant Creation

The purpose of this guide is to describe a guided approach for designing protein variants, mutants, and truncated sequences.

Support users in generating variants of their protein to test on the eProtein Discovery platform and increase their chances to get quickly soluble, functional protein to use downstream in their project.

**Summary - A stepwise guided method for variant creation**

▹ **Step 1 - Identify Relevant UniProt ID**
Use sequence alignment (POI sequence) or direct UniProtID input to identify the starting protein sequence and/or several close protein family members - for example isoforms and splice variants. Annotate each starting sequence with all required metadata.

▹ **Step 2 - Select Candidates**
Filter isoforms, align them and flag functional or structural domains of interest. Our Cloud Software uses AlphaFold to instantly visualize 3D structures, identify key regions for truncation or mutation, and screen variants for soluble expression.

▹ **Step 3 - Rule-based Sequence Editing I**
Apply simple rule based editing for each input Candidate. Depending on the domains present, each input Candidate sequence should generate several “virtual” constructs.

▹ **Step 4 - Rule Based Sequence Editing II - Terminal truncations**
Apply simple rule based editing for each input Candidate. Consider modifications around functional domains of interest, for example removing disordered or unnecessary domains.

▹ **Step 5 - Check for other known stable domains (NMR, X-Ray)**
Identify other important regions and create relevant variants.

▹ **Step 6 - Compile final list of variant Candidates for a POI**

**Details - A stepwise guided method for variant creation**

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| 1 | Identify Relevant Uniprot ID | Sequence or Uniprot ID | Annotated Uniprot sequences | • Identify relevant isoform, canonical isoforms, orthologs, |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | align.<br>• Identify critical domains, Uniprot, Expasy (Structural, functional, etc.) |
| 2 | Select Initial Candidates | Annotated Uniprot sequences | Isoforms and important domains flagged | • Identify relevant isoforms, canonical iso forms, orthologs, align<br>• Identify critical domains, Uniprot, Expasy (Structural, functional, etc.) |
| 3 | Combine | Seqs from | List of input | Combine lists 1 |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | starting list | steps 1 and 2 | Candidates | and 2 |
| 4 | Rule-based SequenceEditing I - identify domains of interest | List of input Candidates | List of Child Candidates 1 Edited sequences named appropriately - rules applied see operations.<br><br>A Child Candidate is a sequence derived from an Initial Candidate by applying Rule-based Editing - Step 4 | If present remove signal peptide from N-terminus<br>If present remove pro-peptide from N-terminus or C-terminus |
| 5 | Rule Based Sequence Editing II - truncations | List of input candidates (Step 3) | | • Consider N-terminal truncation to leave the |

| Step | Title | Input | Output | Operations |
| --- | --- | --- | --- | --- |
| | | + List of Child Candidates (step 4) | | domain of interest. Users may add up to 10 aa upstream.<br>• Consider C-terminal truncation to leave the domain of interest. Users may add up to 10 aa downstream.<br>• Consider both N- and C-terminal truncations to leave the domain of interest. Users may add up to 10 aa upstream or downstream.<br>• Consider truncating to |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | remove disordered regions around functional domain of interest<br>• If available use structure information (X-ray, NMR, AlphaFold) to guide truncation sites |
| 6 | Compile final Screening Candidates | List of input candidates (Step 3)<br>+<br>List of Child Candidates (step 4)<br>+<br>List of | | • Consider your downstream needs (e.g. activity assay, binding assay, structural investigation)<br>• Consider your cartridge format (e.g. a |

| Step | Title | Input | Output | Operations |
| --- | --- | --- | --- | --- |
| | | Child Candidates (step 5) | | FlexiVariant™ or Solubility tag screen<br>• Consider your cartridge budget (e.g. do you plan on running one or more cartridges within this campaign)<br>• Consider Alphafold scoring results |

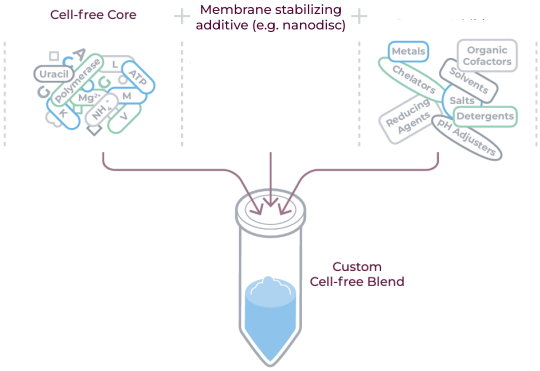
# Additive Selection Guide

Cell-free expression conditions, termed Cell-free Blends, consist of a Cell-free Core reagent supplemented with two additives tailored towards your protein of interest. Nuclera provides standard additives within the Cartridge Reagent Kit (NC3013), but custom additives can also be introduced, provided they are compatible with the system (see [Chemical Compatibility List at https://info.nuclera.com/manual-custom-additives-chemical-compatibility-list.html]).
For membrane proteins, one stabilizing custom additive (e.g., a nanodisc,

which mimics lipid bilayers to support proper folding and function) should be selected, along with either an additional additive from Nuclera's standard additive panel or another compatible custom additive.

Examples of commercially available nanodisc:

| | Human | Mouse | Rat |
|---|---|---|---|
| | MSP1D1dH5 | - | - |
| | MSP1D1 | MSP1D1 | MSP1D1 |
| | MSP1E3D1 | MSP1E3D1 | MSP1E3D1 |
| | MSP2N2 | MSP2N2 | - |
| | His | His | His |
| | No-TAG | - | - |
| | Biotinyl PE | - | - |
| | No-label | No-label | No-label |
| | DMPC | DMPC | DMPC |
| | POPC | POPC | POPC |
| | DMPG | DMPG | DMPG |




| Criteria | MSP1D1dH5 (7-8 nm) | MSP1D1 (9-10 nm) | MSP1E3D1 (12-14 nm) | MSP2N2 (~17 nm) |
|---|---|---|---|---|
| Monomeric GPCRs, small proteins, 1 and 2 transmembrane domains, small dimers | ✔ | ✔ | ✔ | ✔ |
| Small transporters, small dimers | | ✔ | ✔ | ✔ |
| Most proteins except for very big complexes | | | ✔ | ✔ |
| Every protein, not available for biotinylated phospholipids | | | | ✔ |

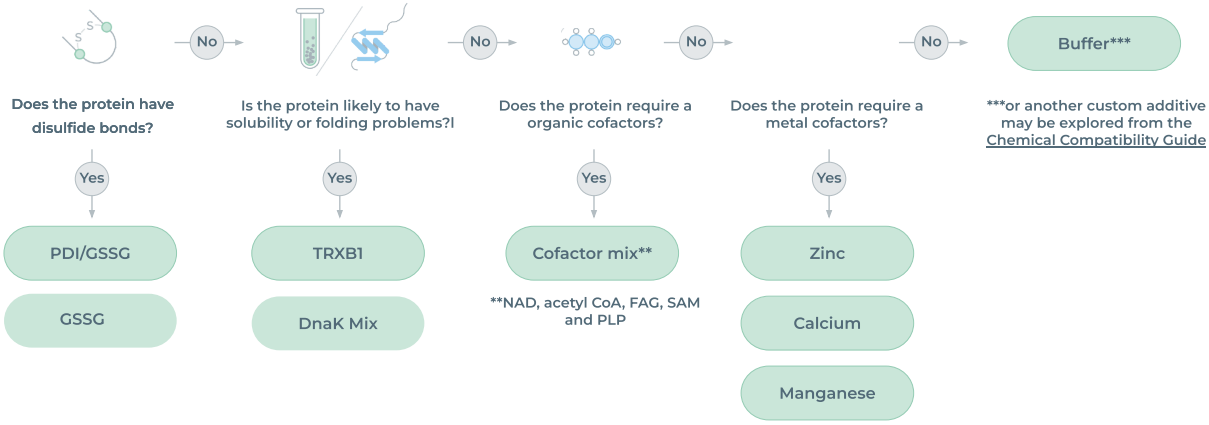
## Step 2: Select between optional tags.

- His-tagged or untagged membrane scaffold proteins for ease of purification if assembling own nanodiscs.
- Preassembled nanodiscs with biotin labeled phospholipids for SPR.

## Step 3: Select a phospholipid.

| Criteria | DMPC | POPC | DMPG |
|---|---|---|---|
| Prokaryotic protein preference | | | ✔ |
| Eukaryotic protein preference | ✔ | ✔ | |
| Eukaryotic preference if interact with negatively charged lipids (GPCRs, Na/K ATPase, ABC transporters and Ion Channels) | | | ✔ |
| Simple and well-defined, suitable for structural and biophysical studies | | | ✔ |
| Physiologically relevant mimic of mammalian plasma membrane | | ✔ | |

## Step 4: Following nanodisc selection, a second additive from Nuclera's standard additive panel or from a custom additive chosen from the Chemical Compatibility Guide can be chosen.




The additives supplied in the Cartridge Reagent Kit NC3013 and their descriptions are listed in Table 1.

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| Additive Buffer | HEPES buffer pH 7.5 and surfactant | CFPS reaction buffer, dilution normalization |
| PDI + GSSG | Protein disulfide | Chaperone and redox modification |

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| Mix | isomerase and oxidized glutathione | to oxidizing environment to support disulfide bond formation |
| TrxB1 | Thioredoxin reductase | Protects proteins from oxidative aggregation and inactivation and acts as a reductase in redox regulation |
| DnaK Mix | Chaperone | DnaK mix Chaperone mix to support folding and prevent aggregation |
| Zinc chloride | Zinc chloride solution | Cofactor that can be required for folding, stability, or activity |
| Calcium chloride | Calcium chloride solution | Cofactor that can be required for compaction, folding, stabilization, or activity |
| Manganese chloride | Manganese chloride solution | Cofactor for metalloenzymes for structure and activity |
| Cofactor Mix | Mix of NAD, acetyl CoA, FAD, SAM, and PLP | Cofactors that assist in folding, stability and activity |
| GSSG | Oxidized | Redox modification to oxidizing |

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| | glutathione | environment |
| 3C protease | 3C protease solution | Protease to cleave off the N-terminal solubility tag at the specific aminoacid sequence (LEVLFQ/GP) |

**Table 1: List of Additives supplied in the Cartridge Reagent Kit NC3010.**

# eProtein Discovery Cloud Software

### Intended Use

The eProtein Discovery Cloud Software, Nuclera's cloud-based software, supports the user in the design and execution of combinatorial protein expression experiments on the eProtein Discovery system.

### Software Updates

▹ Automatic software updates are carried out when instrument is on and not in use with a message displayed on screen.
▹ If needed, automatic update settings and scheduling can be changed in the settings of the instrument (accessible from the side menu). It is recommended to check the time zone is correctly selected.

**On our fully integrated eProtein Discovery system the user can record:**
▹ Sequences of interest.
▹ Constructs compatible with the Nuclera technology.
▹ Recipes for construct expression and scale-up.
▹ Record a specific eGene construct (DNA) containing the sequences of the

proteins of interest and the selection of the eProtein expression conditions. For the design of protein variants, mutants, and truncated sequences, it is recommended to follow the eProtein Discovery Guide for Protein Variant Creation.

## Intended Target User Group

The eProtein Discovery Cloud Software is intended to be used by staff trained to run experiments on the eProtein Discovery Instrument.

**How to access your service**

**Software Requirements:**
Our cloud software works on major browsers (other browsers may also work), without the need to download or install additional packages:
▹ Google Chrome
▹ Mozilla Firefox
▹ Microsoft Edge

**For correct behavior in eProtein Discovery Cloud account, check the following:**
▹ Make sure cookies are enabled in the browser so all parts of our application work as expected.
▹ JavaScript must be enabled in the browser.
▹ It is recommended to set the screen resolution to 1024 x 768 or higher ▹ Check the web browser for add-ons. In some cases, browser add-ons, extensions, ad blockers, or plugins can interfere with our application's functionality.
▹ If you encounter problems consider disabling these extras or try a browser without them.

## Required Files and Other Information

### Files and information needed for the analysis:

▹ A valid amino acid sequence copied as text or a valid DNA sequence copied as text.

## Steps for Access

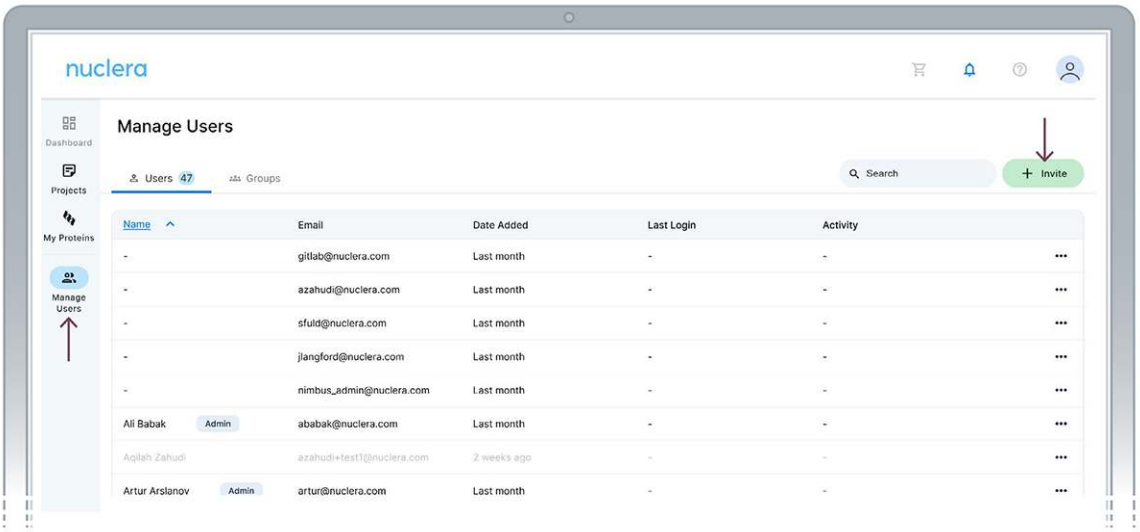
### The following steps are required to access our service

1. The first Administrator user is created by Nuclera.
2. The administrator user will log in to the system and create projects.
3. The administrator user can invite other users and assign them to projects.
4. Non-administrator users will add their details, create password and an instrument access pin at first log in.
5. The admin users can give other users administrator privileges.
6. The admin users can deactivate non-administrator users.
7. After first login users can register proteins and create experiments in their projects

Steps 1-3 are required for first time login. Step 4 is self-service for all users. Steps 6-7 are part of everyday activity on the platform. Steps 2, 3, & 7 are described in detailed below.

### Add a new user - Administrator

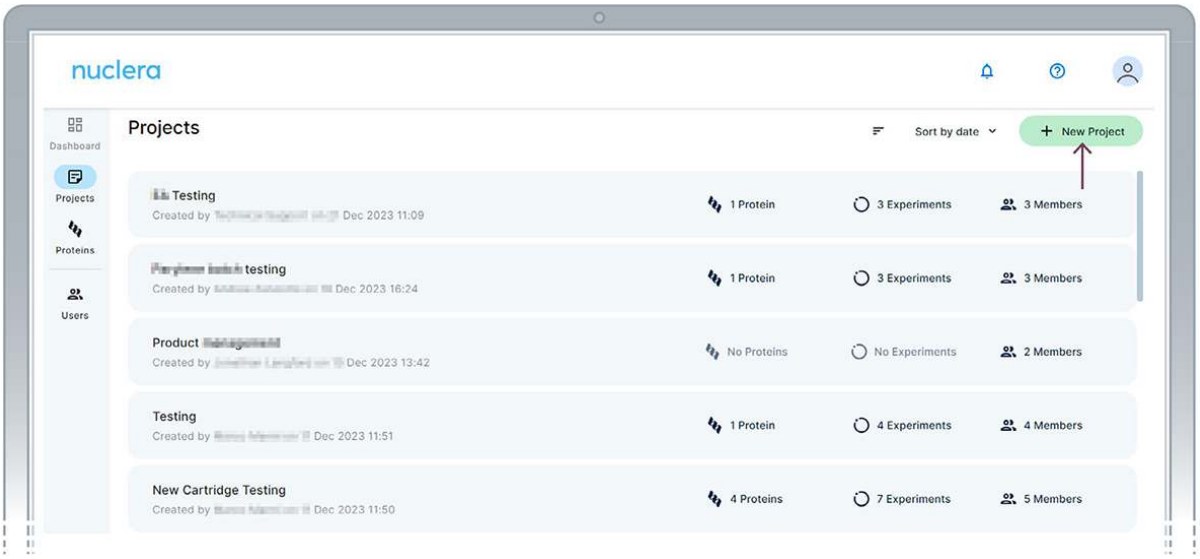
1. From the Manage Users page click the **[Invite]** button.
2. Fill the email address of the invitee and click **[Send]**.
3. The invitee will receive an email with instructions.




## Set up a project - Administrator

In the eProtein Discovery Cloud Software portal, users can create a new project or select from an existing project.

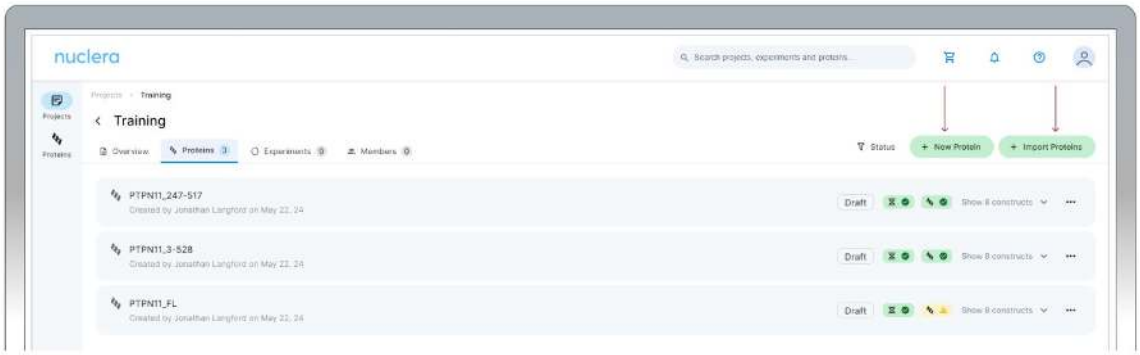
To select an existing project, click on one of the existing projects listed on the screen. To create a new project, click on the **[New Project]** button located on the top right corner of the screen.



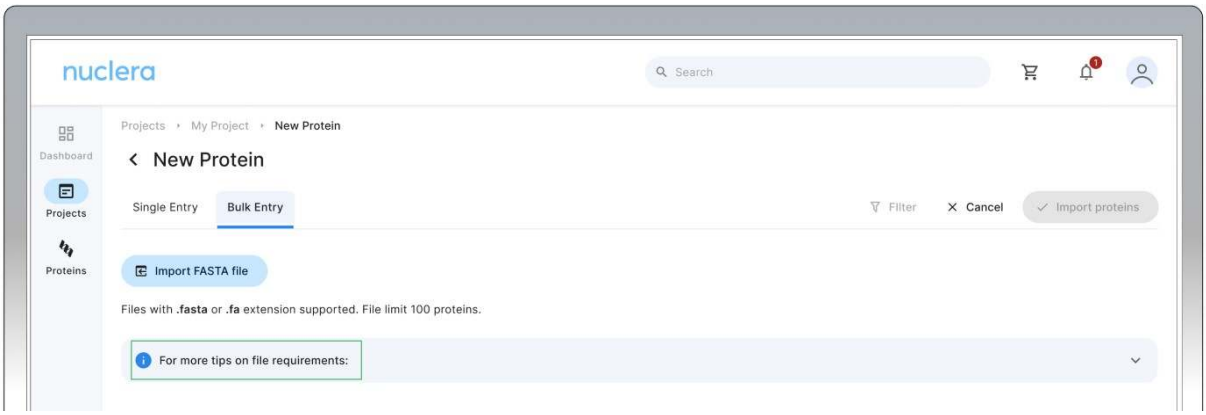
## Register a protein sequence - Any user

Once a project is created, you can navigate through it.

1. Select the **[Proteins]** tab and click the **[+ New Protein]** button.
   Bulk protein sequences can be imported from a FASTA file by clicking on the **[+ Import Proteins]** button. This feature is particularly beneficial for users looking to import 24 sequences or more at once.

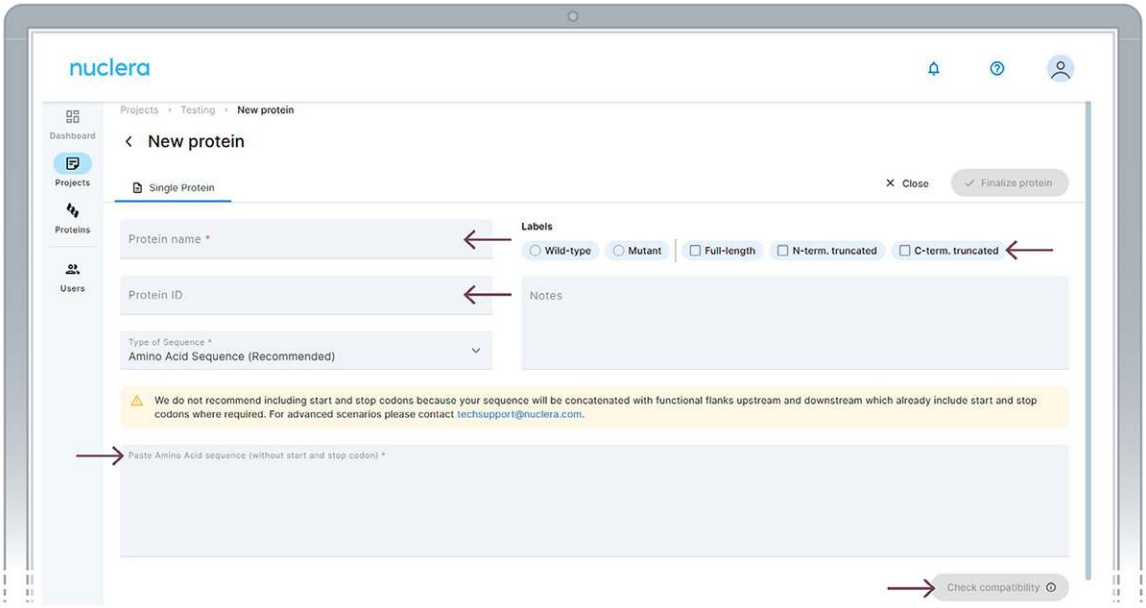



For more information about the **"Import Proteins"** feature, click on the i blue symbol




2. Add a name for your sequence and a reference ID (optional). Select the labels that apply to your protein and add any notes. Select the type of sequence you are submitting, amino acid or DNA. Copy and paste the amino acid or a DNA sequence into the input box. Click on **[Check**

**Compatibility]** and wait until all the tests have been performed.

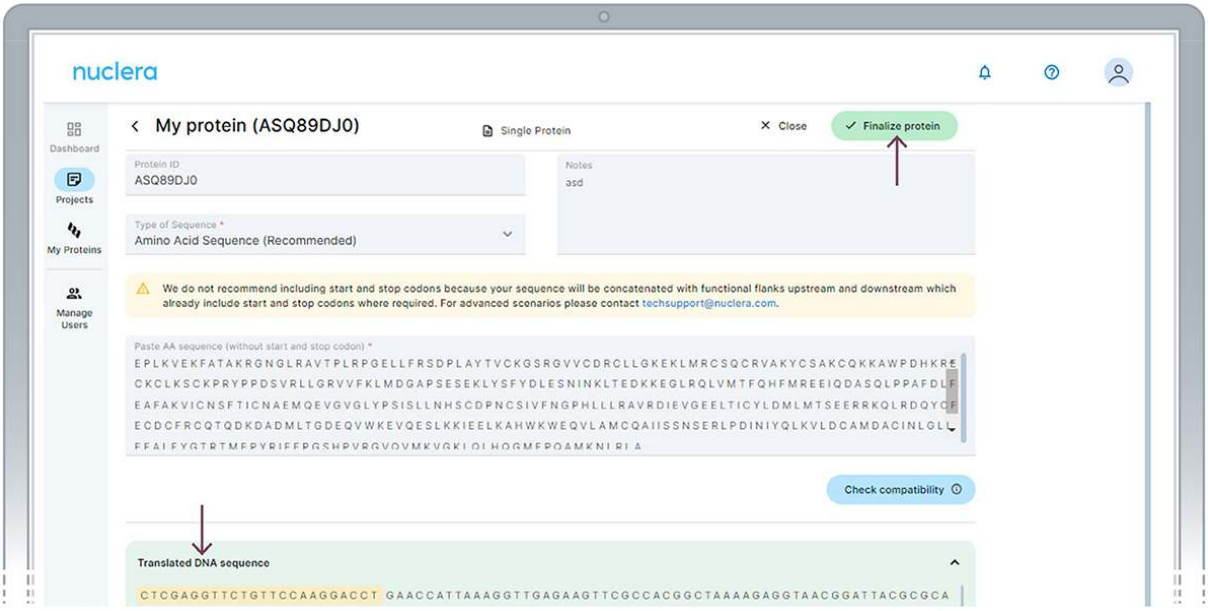



3. If you have uploaded an amino acid sequence, the sequence will be codon optimized and converted to a compatible DNA sequence. Adaptor sequences will also be added to the 5’ and 3’ end. These adaptor sequences will serve as primer annealing points during the PCR reaction to expand each construct with the appropriate fusion tags.

4. The software will perform DNA sequence compatibility checks to ensure that there are no conflicting sequences that can impact DNA synthesis.

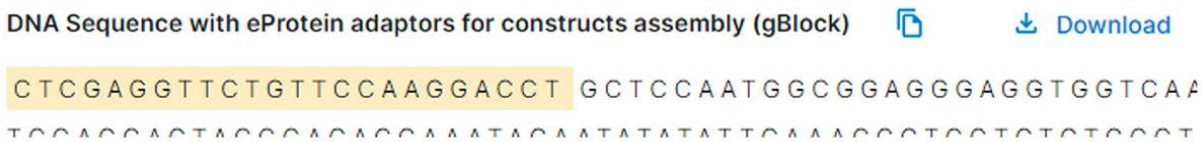
5. Protein expression compatibility checks will also be performed in the background to detect transmembrane domains, disorder regions or the presence of start and stop codons. If any expression incompatibility is detected, a warning signal will be displayed. Users can still proceed at risk or return to modify the sequence.

6. If you are happy with the sequence, press the [Finalize protein] button located at the top right corner of the screen.




7. Once finalized, the protein name or DNA sequence cannot be modified.

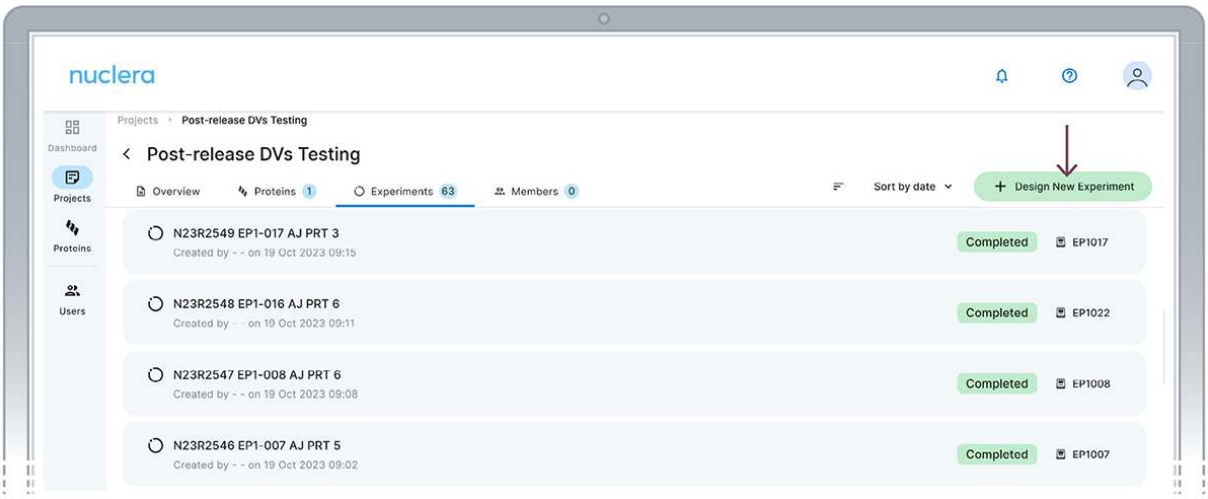
8. The translated DNA sequence can be copied and gene fragment ordered from Nuclera's recommended DNA synthesis vendor.



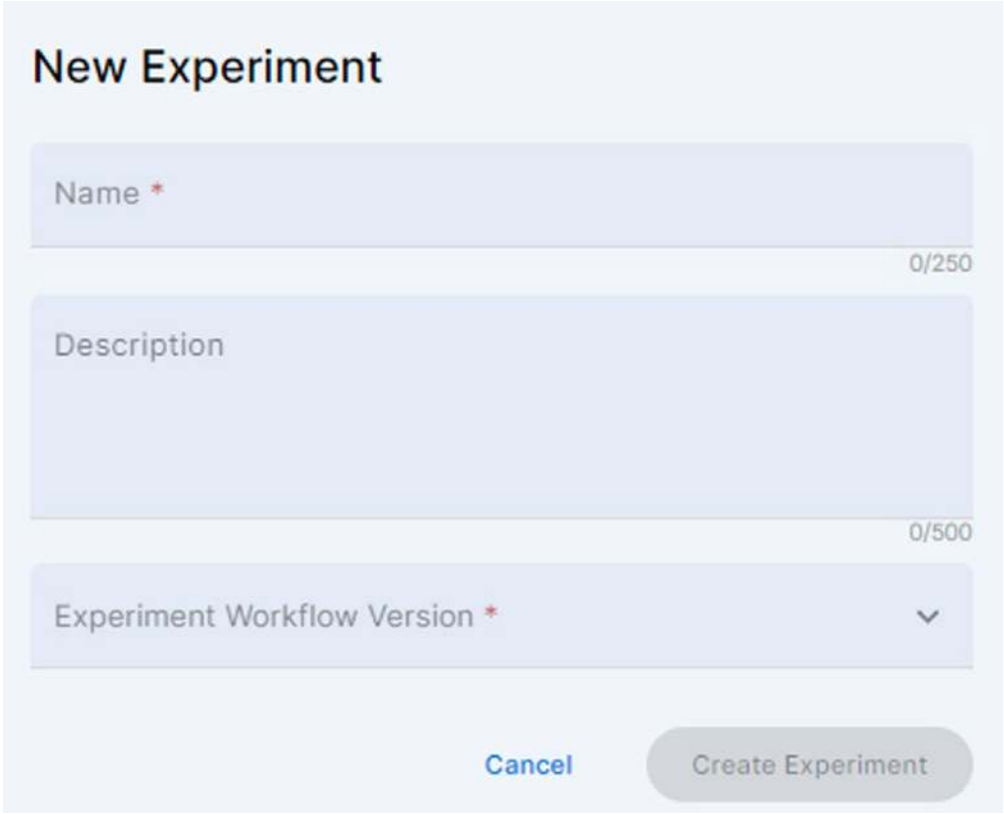

# Design an experiment - Any user

### Expression Screen

1. After registering your protein(s), you can move forward to design your experiment.
2. Select the Experiments tab and click on the **[Design New Experiment]** button.



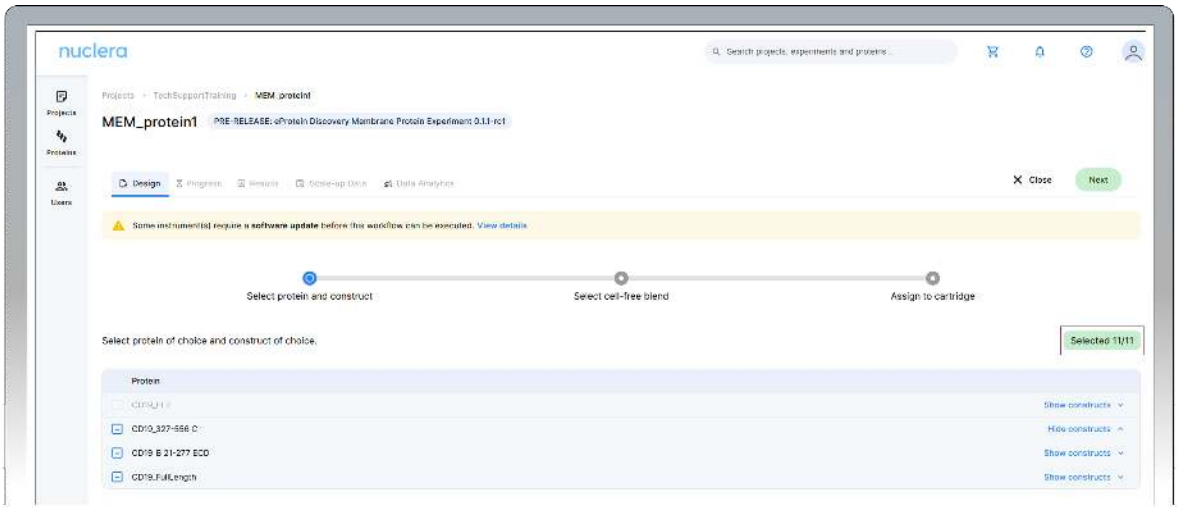

3. Enter the name of the new experiment, add a short text description and select the workflow for **Membrane Protein Screen**. Click the activated **[Create Experiment]** button



**NOTE**

If the version of the Workflow is not compatible with the current version of the Instrument Software, a warning message is displayed, inviting the user to update the Instrument Software.
**Note** On the instrument, an incompatible experiment will appear with a warning sign inviting the user to update the Instrument software."

4. Select 11 DNA constructs to assign to a cartridge. Once a desired number of constructs are selected, click on **[Next]**.
   **Note:** You can load the same construct (duplicate) in two ports if required by your experimental design.



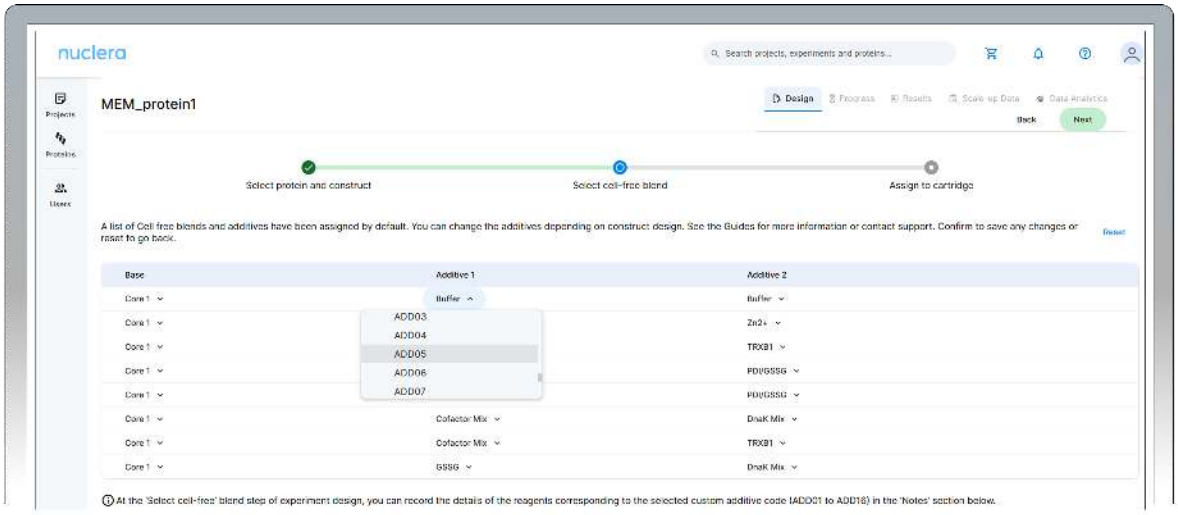

5. Select expression conditions by adding two additives to the Cell-free Core Reagent – The “Additive selection guide” sections of this document will help you to make your selection. If you are undecided on the additive combinations to use, please reach out to Technical Support. Click **[Next]** to proceed.

The eProtein Discovery™ system provides the flexibility to incorporate

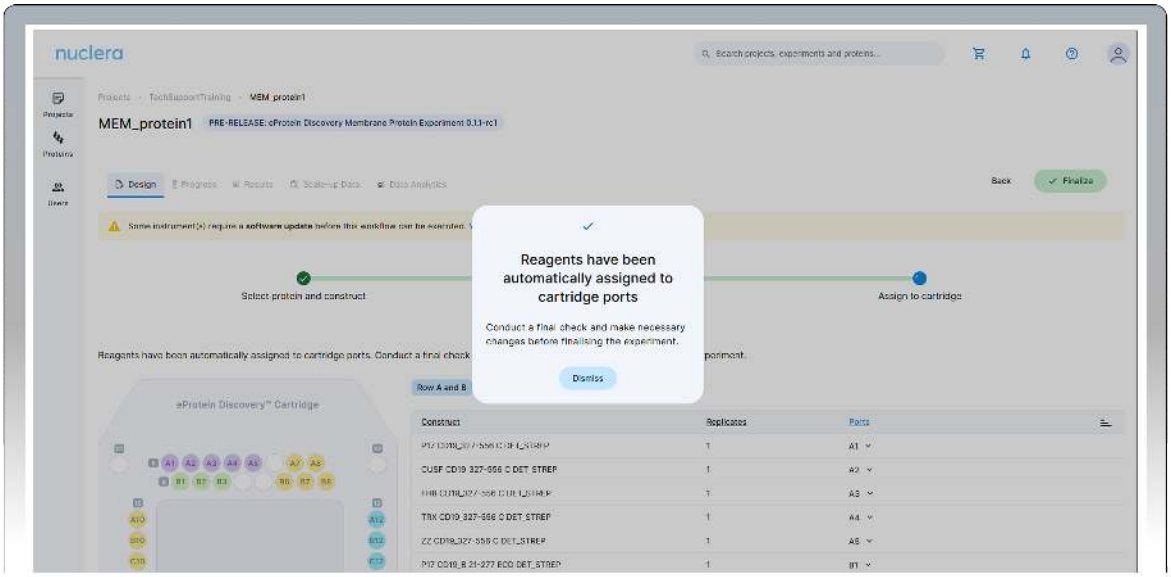
custom additives into expression and purification workflows, enabling users to tailor conditions for unique protein targets. To ensure optimal performance and minimize risks, please consult the Chemical Compatibility List at (https://info.nuclera.com/manual-custom-additives-chemical-compatibility-list.html), which provides detailed guidelines on compatible additives and their maximum allowed concentration. This resource serves as a valuable reference to help you achieve optimal results when working with custom additives. Refer to this before experimenting with custom additives or contact Technical Support if you require more guidance.

**NOTE**

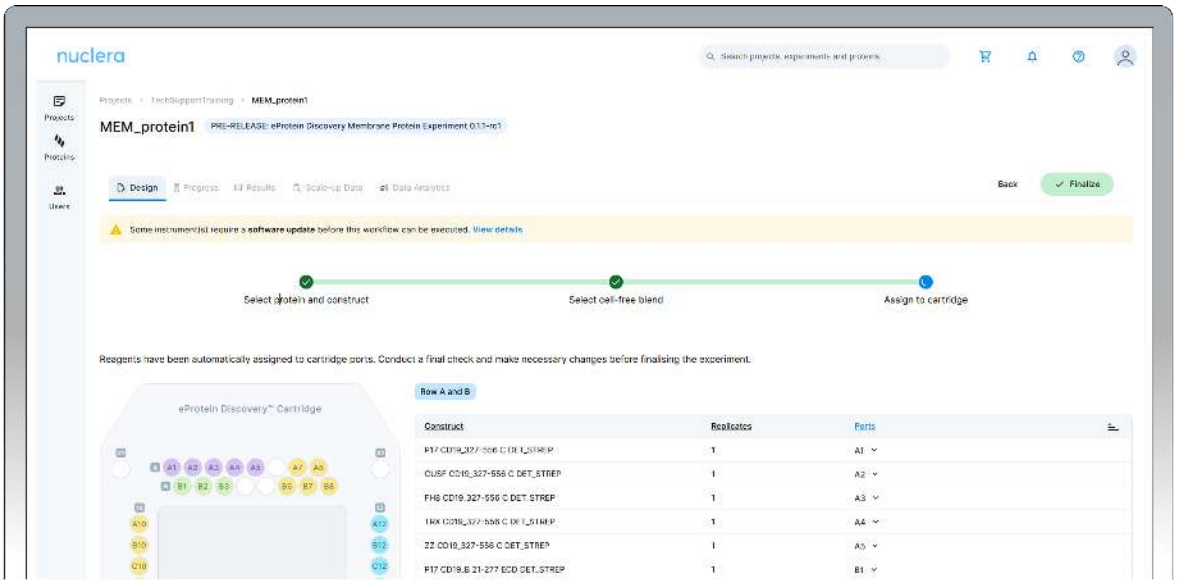
An additive can be selected twice to enhance a specific condition.
If using an additive that is included in our standards Cartridge Reagent Kit, use the dropdown menu to select ADD01, 02, 03 etc to distinguish between different type of nanodisc.



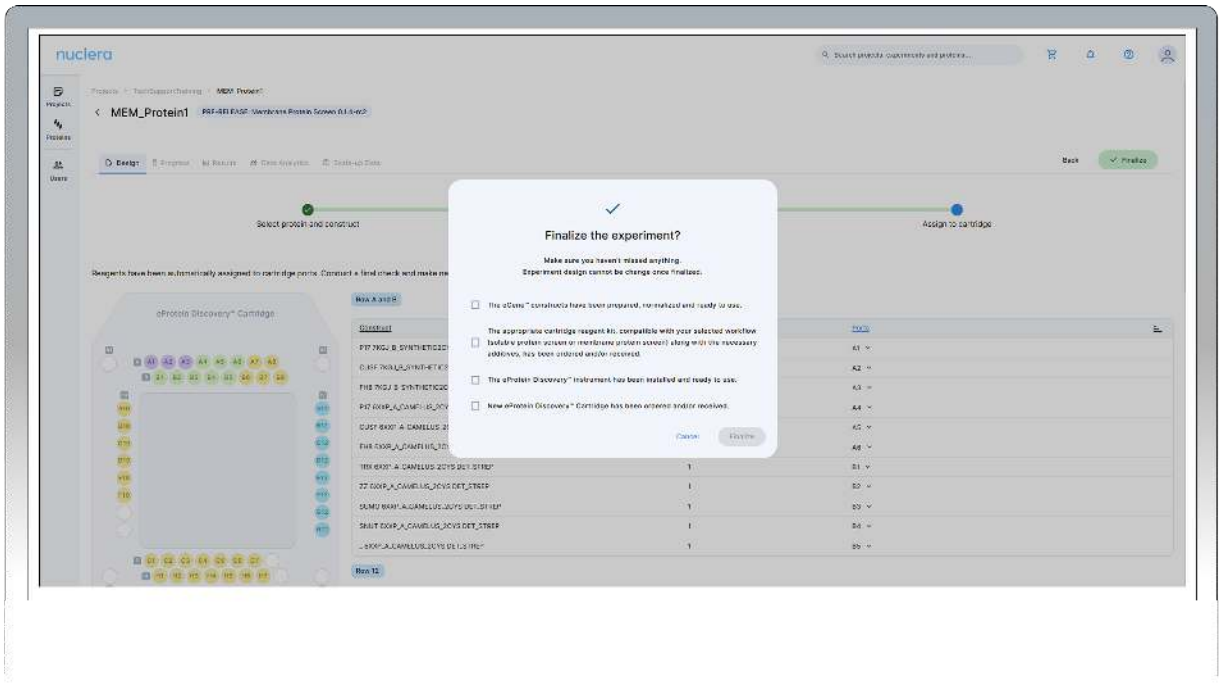

6. All samples and reagents are now assigned to a specific port on the cartridge.




7. Review & Correct sample allocation – In the final step you have the opportunity to inspect and potentially change port location for DNA samples and Cell-free Blends. Drag & drop in the interactive cartridge map or use the port menu available to every sample. Press the **[Next]** button to proceed.



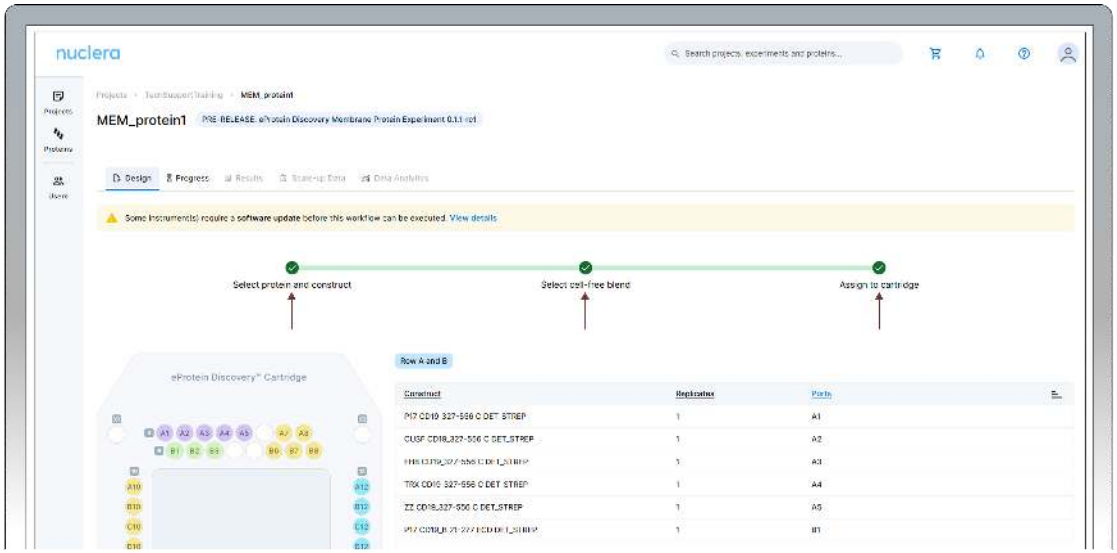
8. Press the **[Finalize]** button to proceed. This will prompt to a checklist to make sure everything is ready for the experiment.




**note:** Once finalized, the experimental design cannot be changed.

Your experiment is now available on the instrument.

9. A summary of your experiment is now available. You can toggle and review the various aspects of your experiments by selecting the Design tab and clicking any of the **[three green circles]** in the design page.




# Preparation of the eProtein Discovery reagents

The preparation of the reagents takes about 1 hour.

### Connect the vial of base fluid to the pump module

▹ In anticipation of starting a new experiment, take a fresh vial of base fluid, open it, and connect it to the left holder on the eProtein Discovery instrument pump module (Figures 3 and 4).

> **IMPORTANT NOTE**
>
> It is important to equilibrate the base fluid with the lab atmosphere prior to use. This is to prevent outgassing of the base fluid during the run, as air bubbles can interfere with the droplet movement. **We recommend attaching the base fluid to the instrument the day before you will perform the run. An acceptable alternative is to**

> **incubate the uncapped base fluid at 30°C / 86°F for 1 hour**.

▹ Connect the waste container, empty, to the right holder of the pump module (Figures 3 and 4).

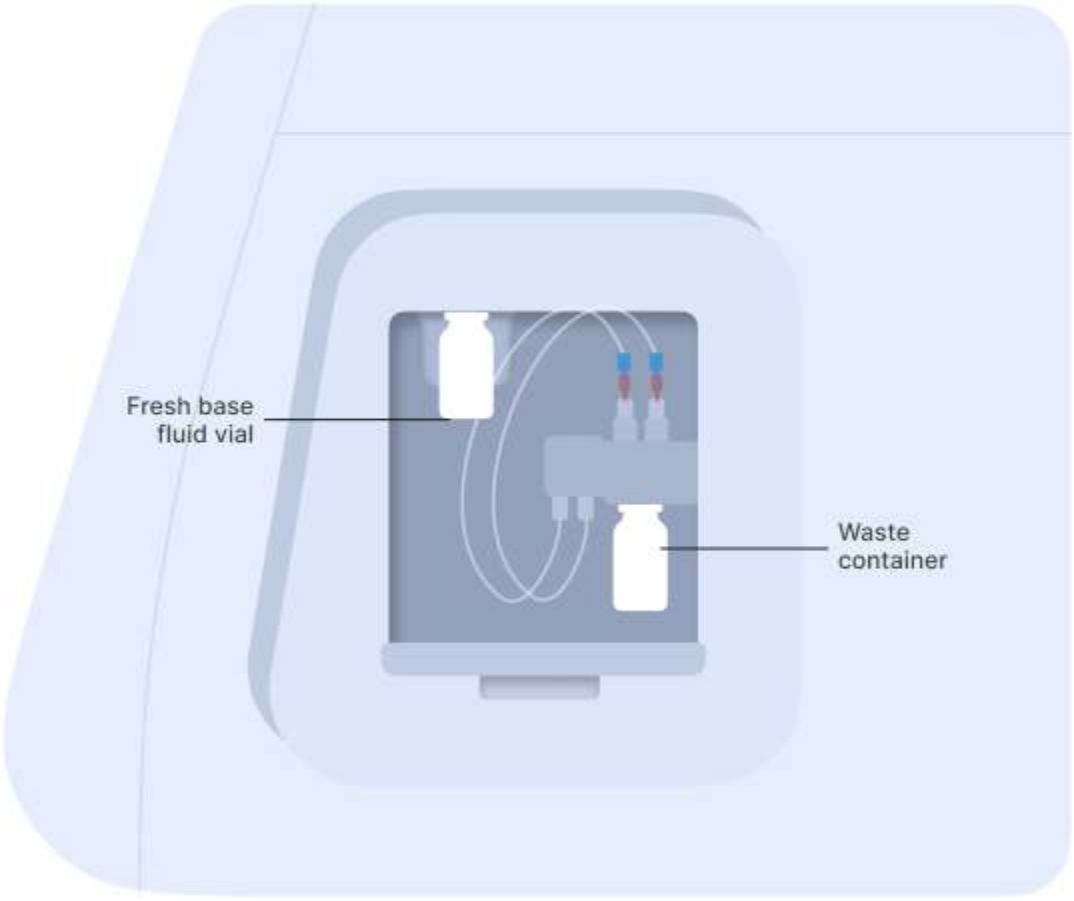



**Figure 3: Vial of base fluid and the waste container connected to the pump as shown on the screen**

[1]  [2]

**Figure 4: Connection of the empty waste container [1] and the vial of base fluid [2] to the pump**

## Prepare the transfer plate

After connecting the base fluid to the holder on the instrument, take all the reagents out of the freezer.

The eProtein Discovery reagents need to be prepared and loaded onto a 96-well transfer plate following the layout and volumes in Figure 5 and Table 2.
**Note:** It is critical to follow this layout exactly because it determines how the reagents are dispensed in the eProtein Discovery cartridge.

**IMPORTANT NOTE**

It is critical not to leave any port empty. If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit, **not with water.**

**Tip:** Empty ports can be used for duplicates.

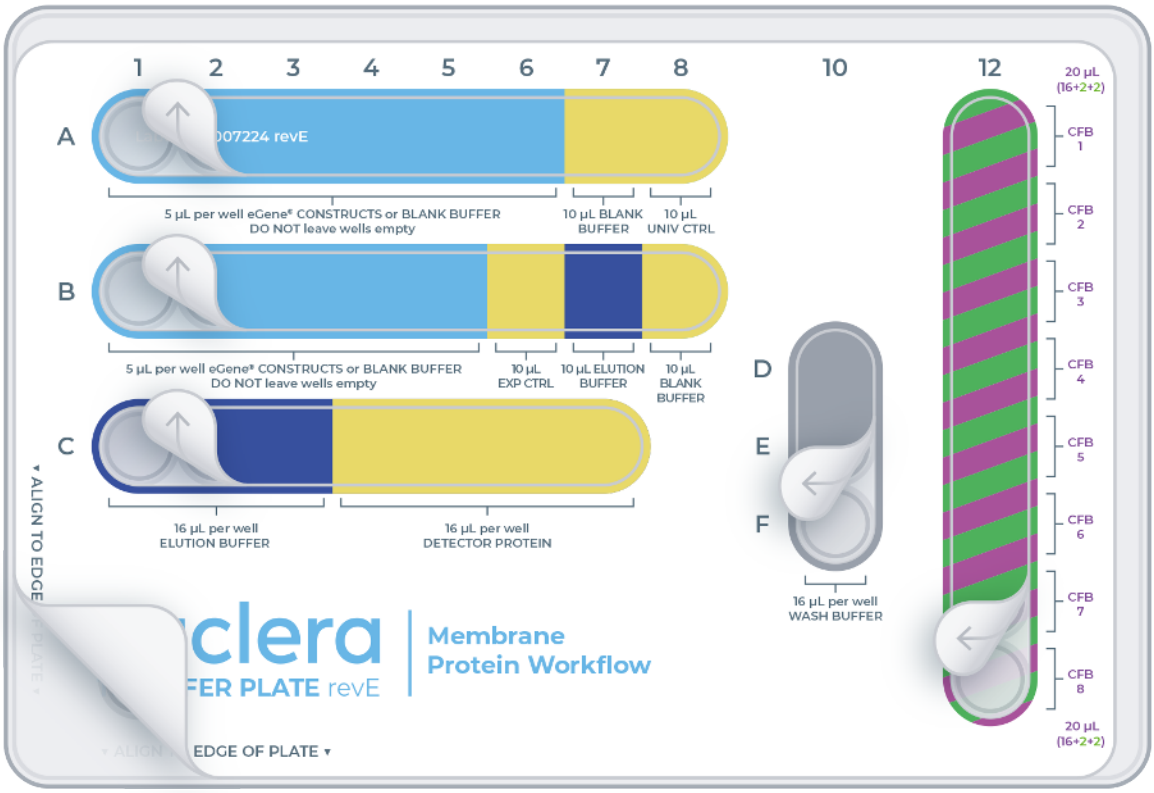



**Figure 5: Transfer plate layout**

| Reagent | Volume (µL) |
|---|---|
| eGene construct | 5 |
| Controls: Blank Buffer, Universal Control (Univ. Ctrl), | 10 |

| Reagent | Volume (µL) |
| --- | --- |
| Expression Control (Exp Ctrl) | |
| Cell-free Blend (CFB): Cell-free Core Reagent + Additive 1 + Additive 2 | 20 (16+2+2) |
| Wash Buffer (Wash Buffer) | 16 |
| Elution Buffer (Elut. Buffer) | 10 µL B7, 16 µL in C1,2,3 |
| Detector Protein (Det. Prot.) | 16 |

**Table 2: Reagents and volumes to load on the transfer plate**

**1.Take the Strep Beads from the fridge and the Cartridge Kit reagents (box with the purple stripe on the label) from the -80°C freezer.**


**2.Place an empty 96-well transfer plate on ice.**

Apply the transfer plate sticker provided in the Cartridge Reagent kit and place the 96-well plate on ice.

> **NOTE**
>
> The transfer plate should be kept on ice until the transfer of reagents to the Cartridge.
>
> Ensure you prepare the Cell-free Blends last.
>
> Be careful not to introduce any ice into the wells

### 3. eGene constructs (DNA)

Take the vials or the plates with the eGene constructs made in advance using the eGene Prep Kit out of the freezer and thaw on the benchtop at room temperature. This takes approximately 15 minutes.
**Note:** the vials or the plates should be centrifuged for a few seconds to ensure all the liquid is at the bottom of the wells.

> ⚠ **IMPORTANT NOTE**
>
> It is critical not to leave any port empty. If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit, **not with water.**
>
> **Tip:** Empty ports can be used for duplicates.

For every eGene, load 5 µL into the selected well:
▹ A1 to A6
▹ B1 to B5

**Note:** It is critical to load the eGene constructs onto the transfer plate in the exact order that they have been finalized in the experiment planned in the eProtein Discovery Cloud Software.

### 4. eProtein Discovery purification reagents

Thaw the Wash Buffer and the Elution Buffer on the benchtop at room temperature. This will take about 20 minutes.
▹ Load 16 µL of Wash Buffer into wells D10, E10 and F10
▹ Load 16 µL of Elution Buffer into wells C1, C2, C3
▹ Load 10 µL of Elution Buffer into well B7

### 5. eProtein Discovery controls

From the kit kept at -80°C, take the controls out and thaw them on ice.
▹ Load 10 µL of Blank Buffer into wells A7 and B8.

▹ Load 10 µL of Universal Control into well A8.
▹ Load 10 µL of Expression Control into well B6

**6. Strep Purification Beads**

Strep Purification Beads are provided in 2x 200 µL aliquots of 5% v/v suspension – To prepare the Strep Beads:

1. Take the 2x vials of Strep Beads from the fridge and give them a quick spin for 2 seconds in a microcentrifuge to ensure all material is collected at the bottom of the tubes.
2. Resuspend the beads by gently pipetting up and down 10 times with a p200 pipette set on 90 µL.
3. Transfer 3x 90µL (2x 90 from one tube and 1x 90 from the second tube) of the resuspended beads into a 3x 1.5 mL tubes. Beads settle quickly - be sure to resuspend between aliquots. Discard the rest only after the experiment starts, in case more volume is required.
4. Place the three tubes with Strep Beads on a magnetic particle separator and capture for 1 min.
5. Remove all the supernatant with a p200 pipette and discard the liquid.
6. Remove the three tubes with Strep Beads from the magnetic particle separator. Resuspend the beads in 100 µL Wash Buffer by slowly pipetting up and down 10 times.
7. Repeat steps 4 to 6 twice more for a total of three washes.
8. After the third wash, spin down the three tubes and place it back on a magnetic particle separator and capture for 1 min.
9. Remove all the supernatant with a p200 pipette and discard the liquid.
10. Spin down the tube, place it back on a magnetic particle separator and remove the residual buffer with a p20 pipette.
11. With a p20 pipette, resuspend the beads in the three tubes with 10.5 µL of Wash Buffer by gently pipetting up and down 10 times to create three beads solutions of 15 µL at 30% v/v Strep Beads working solution

12. Keep the beads in the tube on the bench, not on ice.
    **Note:** The beads should NOT be loaded onto the transfer plate.

**7. Detector Protein**

Spin down the tubes for 2 seconds to collect the full volume at the bottom. Load 16 µL of Detector Protein into wells C4, C5, C6 and C7 of the transfer plate as instructed on transfer plate label.

**8. Preparation of the Cell-free Blends**

For each expression screening experiment, up to eight distinct 20 µL Cell-free Blends can be prepared by combining 16 µL of Cell-free Core Reagent with 2 µL of a first additive and 2 µL of a second additive. If fewer combinations are used, fill the remaining wells of Column 12 of the transfer plate using 16 µL of Cell-free core + 4 µL of Additive Buffer.

> **NOTE**
>
> The total volume of blend should always be 20 µL final.
> Ensure that the Cell-free blend is thoroughly resuspended by pipetting up and down from near the bottom.

1. Thaw Cell-free Core Reagents and Additives on ice
2. Once the Cell-free Core reagents and Additives are thawed, vortex each for 2 seconds to ensure they are well mixed.
3. Centrifuge for 2 seconds the Cell-free Core reagents and Additives using a microcentrifuge to return any droplets to the bulk aliquot.
4. Add 16 µL of Cell-free Core reagent to wells A12-H12.
5. Add 2 µL of your first selected additive to wells A12-H12.
6. Add 2 µL of your second selected additive to wells A12-H12.

> **NOTE**

It is critical to load the Cell-free Blends onto the transfer plate in the exact order that they have been finalized in the experiment planned in the eProtein Discovery Cloud Software.
Ensure the Cell-free Blend is thoroughly resuspended with the chosen additives by pipetting up and down near the bottom of the tube, making sure that any viscous components are fully mixed.

## Set up the experiment on the instrument

### Log in and select the experiment

1. Press the **[Power Switch]** to activate the Instrument power-up, initialization and self-test sequence.
2. Log into the instrument software by selecting the user and entering PIN (Figure 6a and 6b).

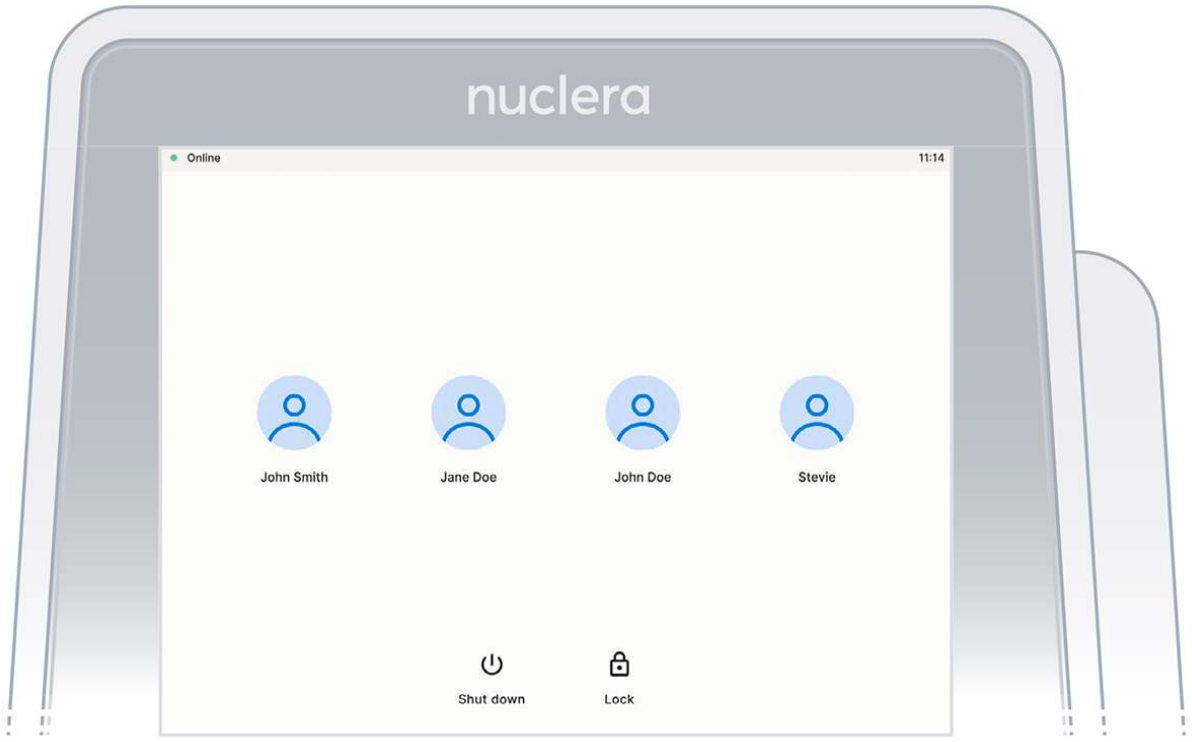


**Figure 6a: User accounts on the instrument software**

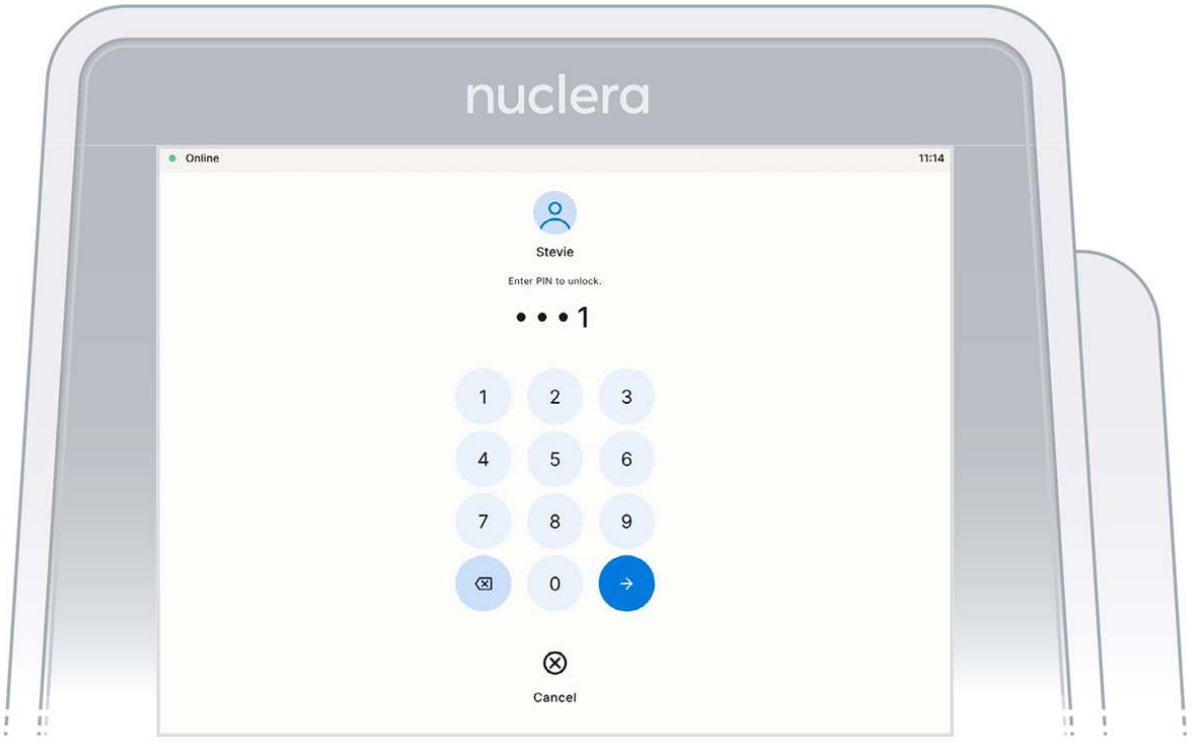



**Figure 6b: PIN Interface**

3. On the instrument software, select an experiment you set up on eProtein Discovery (Figure 7).

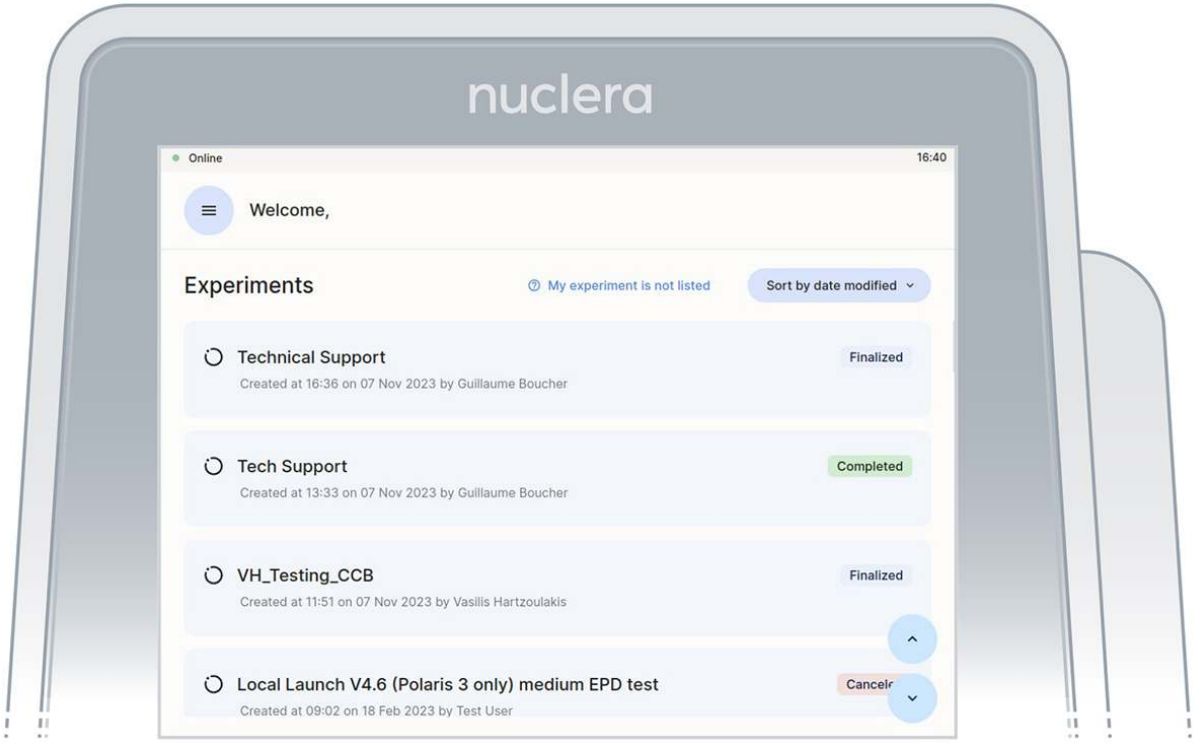



**Figure 7: Instrument software welcome page with the list of finalized experiments**

4. Read the **Before you proceed** section and press the **[Next]** button (Figure 8).

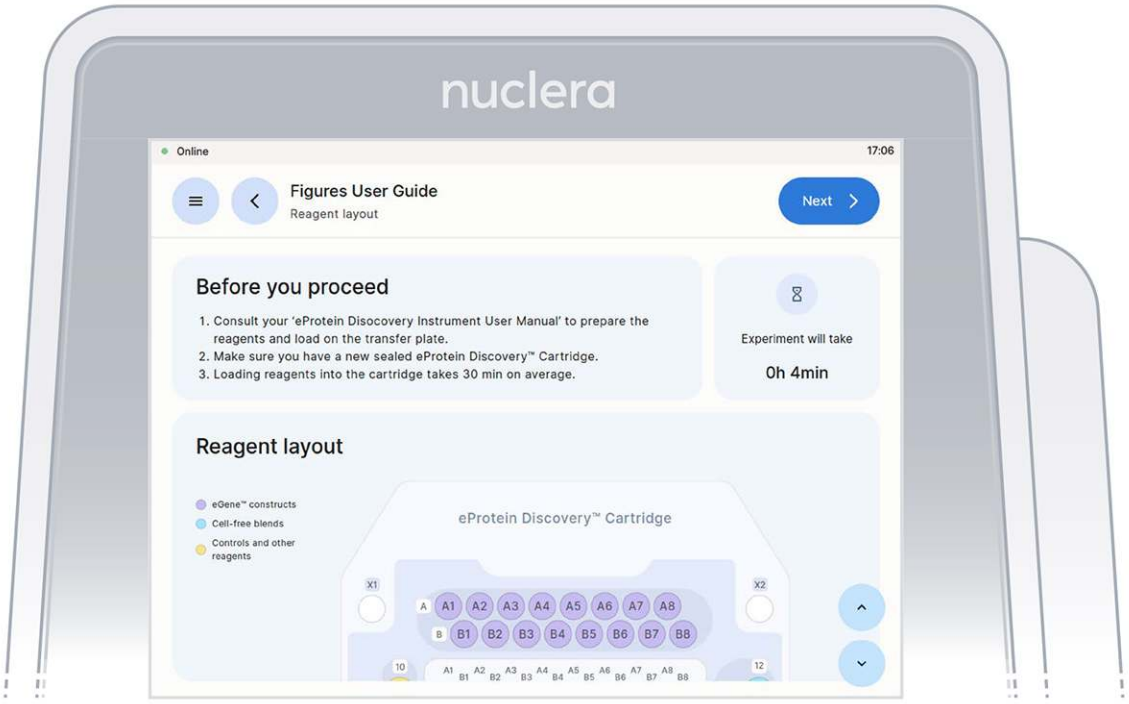



**Figure 8: Instrument software welcome page with the list of finalized experiments**

5. Go through and tick the checklist, and press the **[Next]** button (Figure 9). The drawer will open.

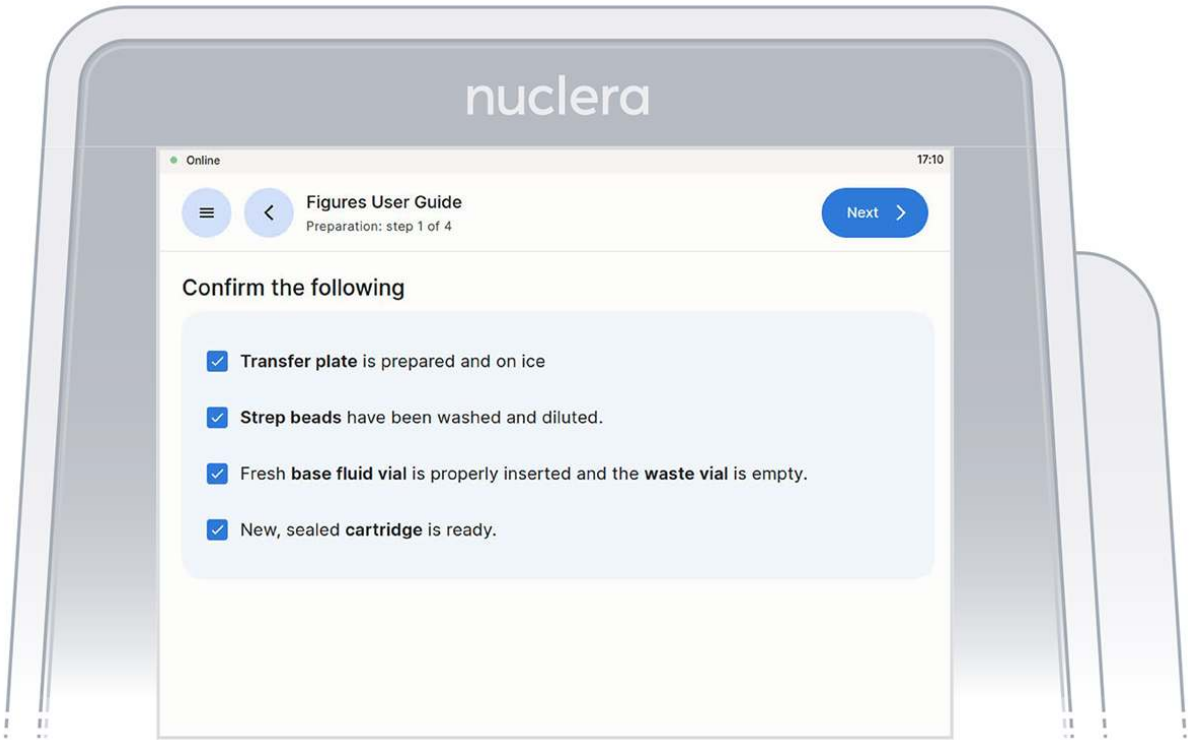



**Figure 9: Loading of the cartridge on the eProtein Discovery instrument.**

6. Unpack and load a cartridge as shown on the screen of the eProtein Discovery instrument
7. Place the cover on the cartridge, avoid touching the electrical connectors, and press the **[Next]** button (Figure 10).


**Figure 10: Checklist screen before the experiment starts.**

**Note:** keep the cartridge packaging to dispose of the cartridge after use.

8. Keep cover on the cartridge. Markings on the cover will guide you through the loading process.

## Set up the pump on the instrument

**Follow the on-screen instructions to complete the experiment.**

▹ These instructions will guide you in operating the eProtein Discovery instrument and completing an experiment on the instrument.

▹ The instructions must be followed in the order shown on the screen.

▹ You can navigate forward and back through the steps using the buttons at the top.

▹ You can scroll up and down using the arrows at the bottom right of the screen when shown or with your fingers.

**Note:** once you start the experiment, the back button on the instrument will be disabled.

1. On the right hand side of the instrument, ensure the tubings for the integrated pump are placed in the tubes holder, and press the **[Next]** button (Figure 11).


**Figure 11: Verification screen that the tubings for the integrated pump are placed in the tubing holder.**

2. Ensure the vial of base fluid and the waste container have been connected to the pump located on the right hand side of the instrument. Press the **[Next]** button (Figures 12).

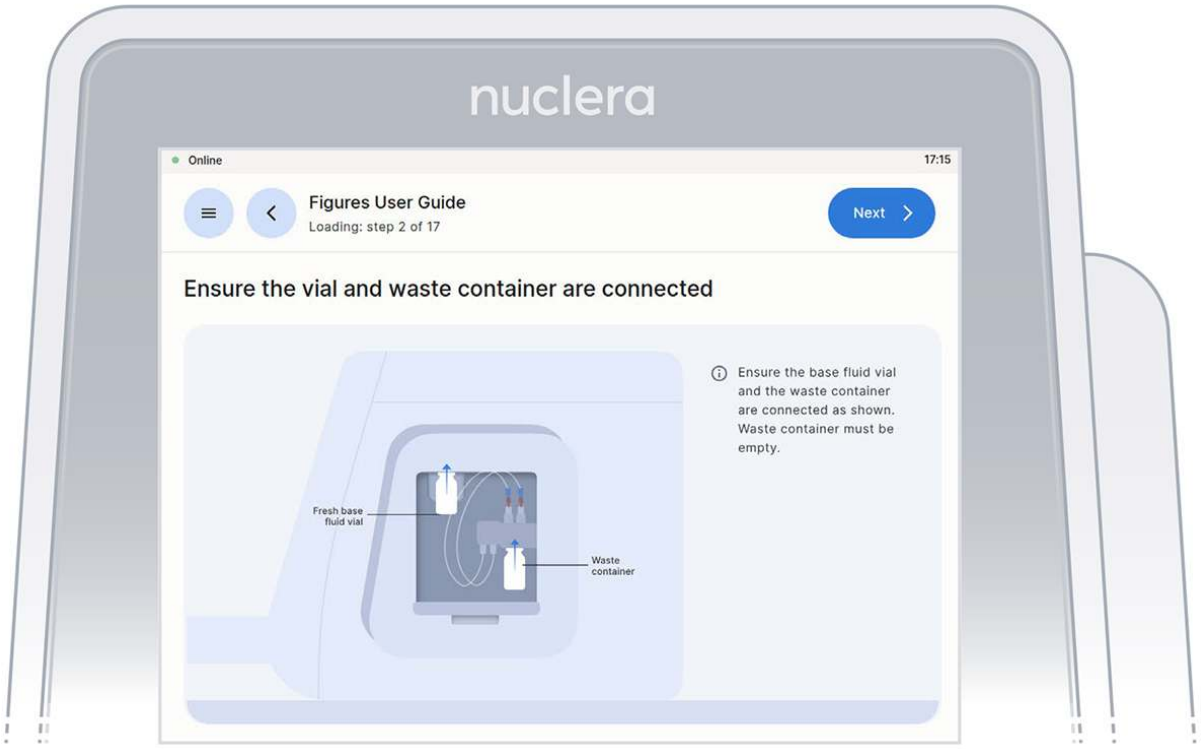



**Figure 12: Vial of base fluid and the waste container connected to the pump as shown on the screen**

## Filling the cartridge with base fluid

1. With the tubes and containers in place, ensure that some of the base fluid has dripped into the waste container (Figure 13).


**Figure 13: Priming the pump tubes with base fluid**

2. Remove the tube connectors from the holder, connect them tightly to the corner ports X2 and X3 of the cartridge, and press the **[Next]** button (Figure 14). Either connector can be interchangeably inserted into corner port X2 or X3.

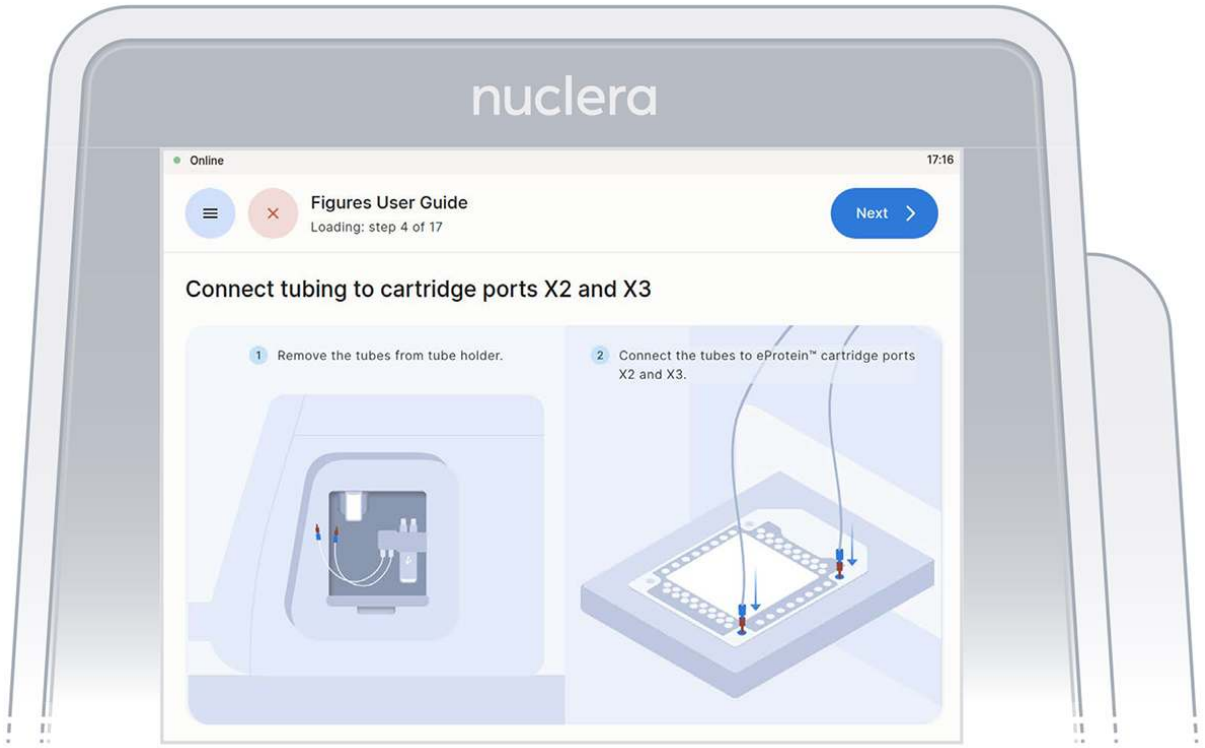



**Figure 14: Inspection that all the ports on the cartridge are filled with base fluid**

3. After the base fluid has loaded, inspect the cartridge for air bubbles that may have been introduced during the priming with base fluid. If any air bubbles persist after base fluid priming, use a single-channel p200 pipette to aspirate the air bubbles from the nearest port and slowly reinject the base fluid that was aspirated into a corner port (X1 or X4). Press the **[Next]** button (Figure 15).

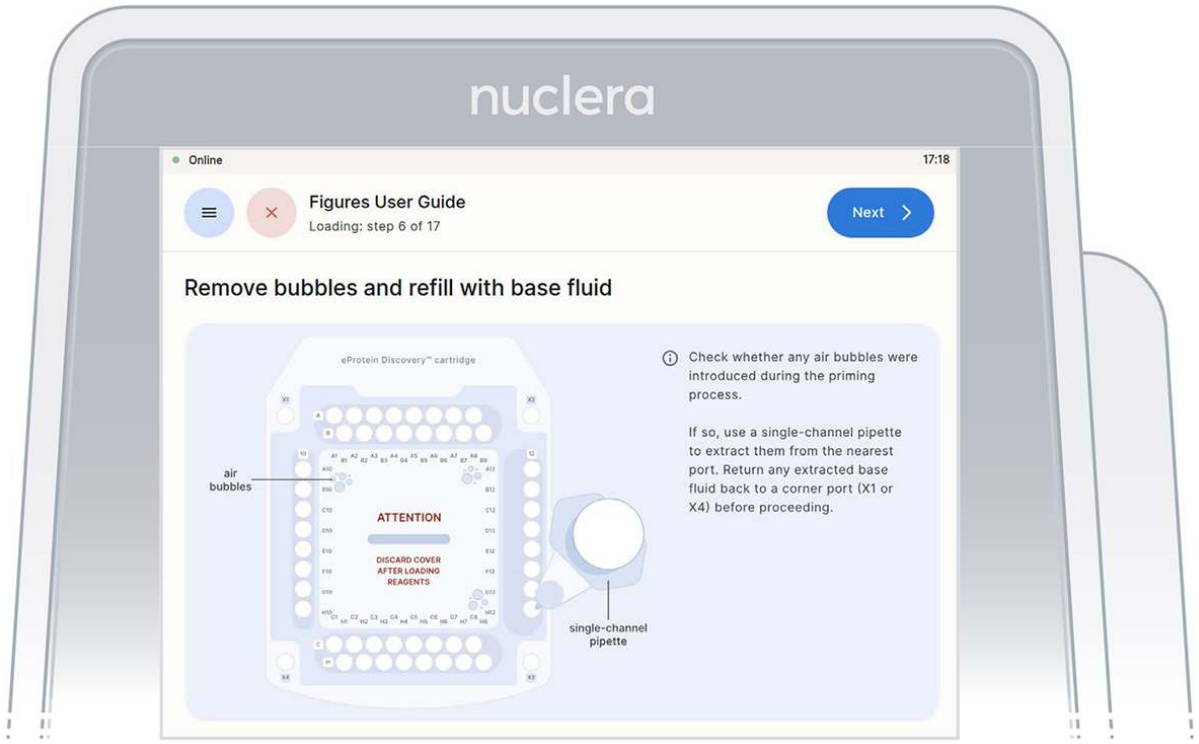



**Figure 15: Connection of the pump tubes to the cartridge**

4. Inspect the ports on the cartridge after the priming with base fluid is complete. Ensure all the ports are filled and press the **[Next]** button (Figure 16).

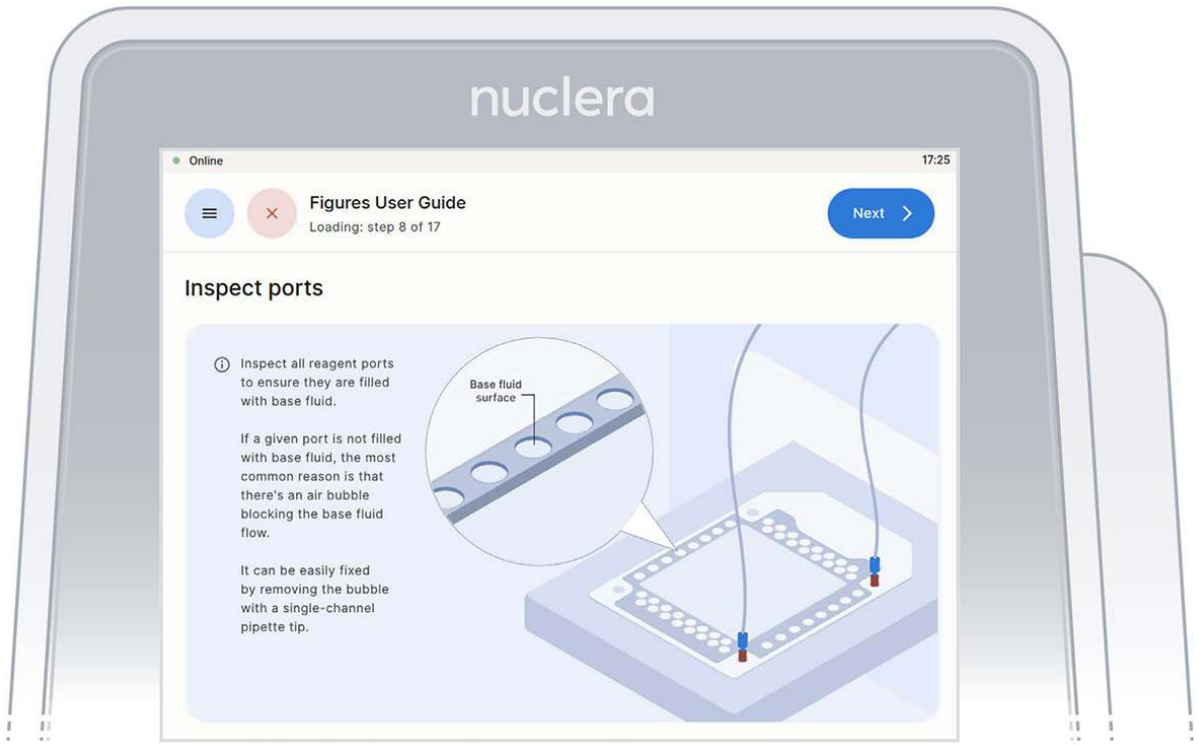



**Figure 16: Confirm that all the ports on the cartridge are filled with base fluid**

5. Remove the cartridge cover if used during the loading (Figure 17)

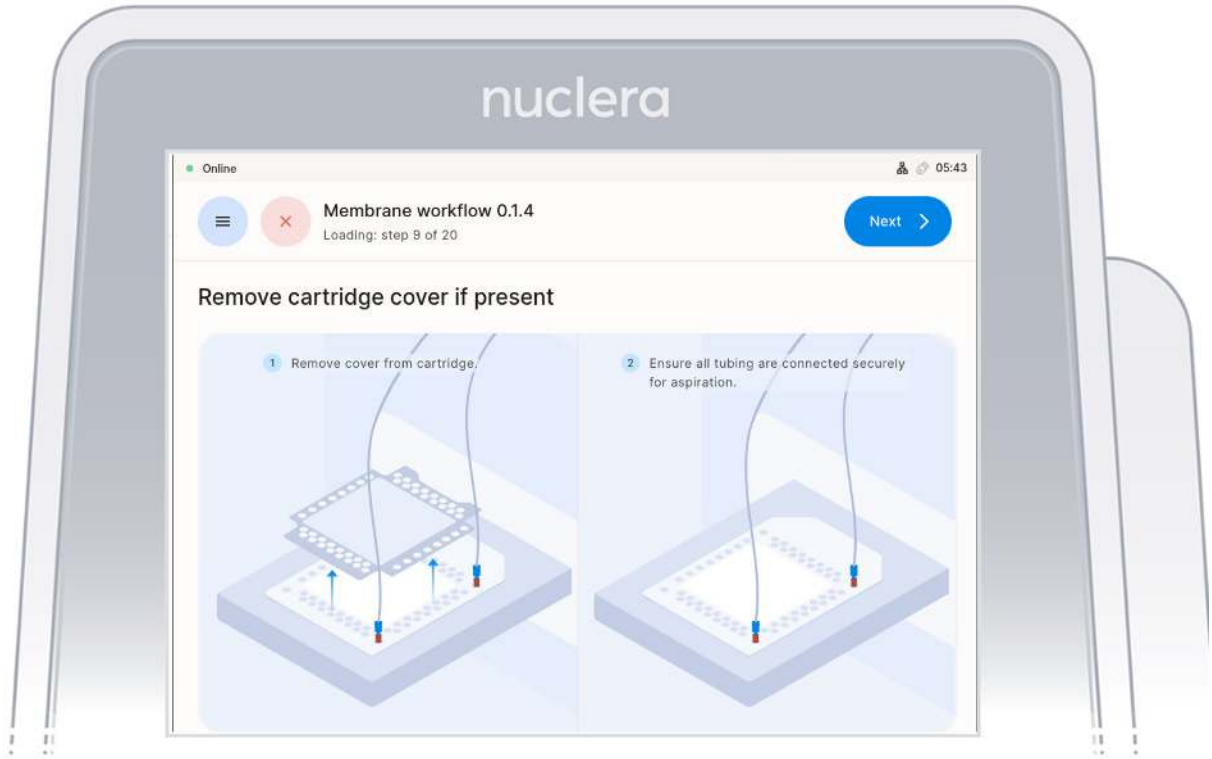



**Figure 17:Remove cartridge cover**

# Load the reagents on the cartridge

**Tips for a perfect loading:**

▹ Follow the on-screen instructions that will guide you in loading the reagents.

▹ The loading of the reagents should be done using an 8-channel pipette.

▹ To facilitate the pipetting of the reagents, the transfer plate can be moved from the ice bucket to the bench.

▹ Check the plate for the presence of air bubbles. Air bubbles can be removed by spinning the plate in a swing rotor centrifuge for about 10 seconds. Seal the plate before using a plate centrifuge

▹ After aspirating the reagents, make sure that all pipette tips are filled evenly, and contain no air bubbles.

▹ Ensure the pipette tips are positioned just below the surface of the base

fluid, away from the sides and bottom of the port.
▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.
▹ Do not engage the pipette tips fully into the ports, the tip ends should not touch the bottom of the ports while dispensing the reagents (Figure 17).

> **NOTE**
>
> ▹ **Do not pass the first stop** as it would release air bubbles

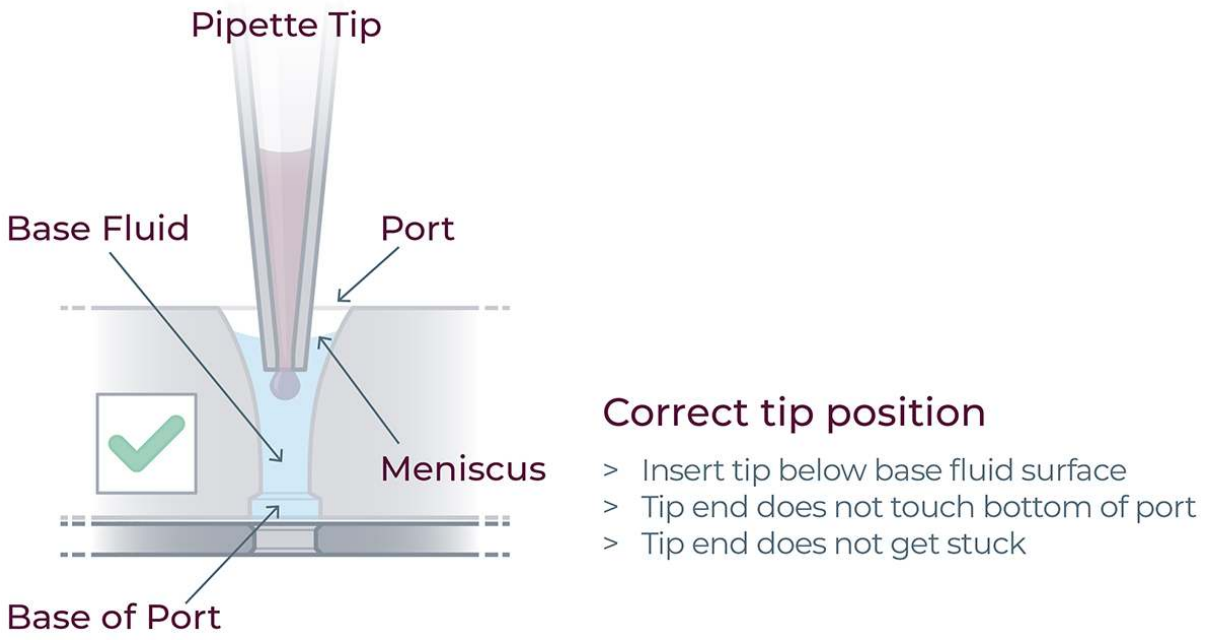



**Figure 17: For correct reagent loading the pipette tip is immersed in the base fluid and not touching the bottom of the port**

## Load reagents

> **IMPORTANT NOTE**

It is critical not to leave any port empty. If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit, **not with water.**

**Tip:** Empty ports can be used for duplicates.

**1. Reagents - row A:**

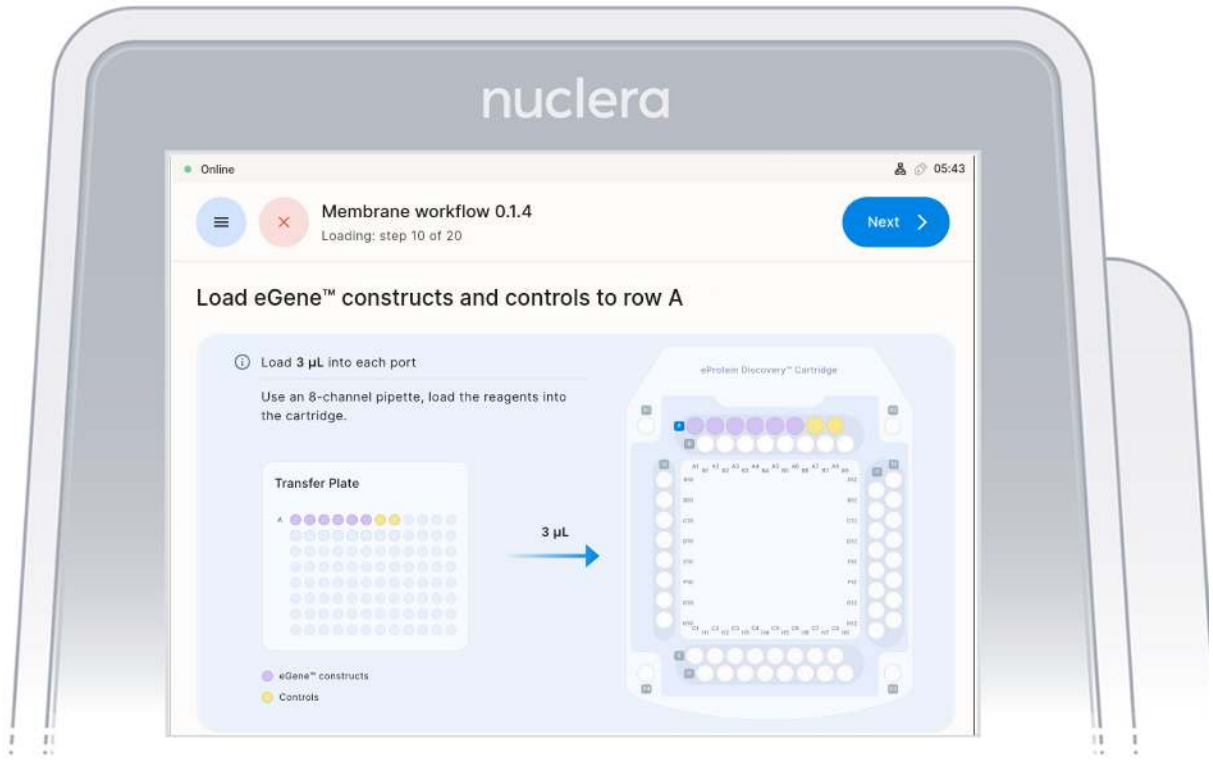
▹ Load x8 fresh p20 pipette tips and aspirate 3 µL of the reagents from the transfer plate wells A1-A8 into ports A1-A8 of the cartridge (Figure 21).

▹ Ensure the pipette tips are positioned just below the surface of the base fluid, away from the sides and bottom of the port.

▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.

▹ Eject the pipette tips into a waste container.

▹ Press the **[Next]** button on the screen.



**2. Reagents - row B:**

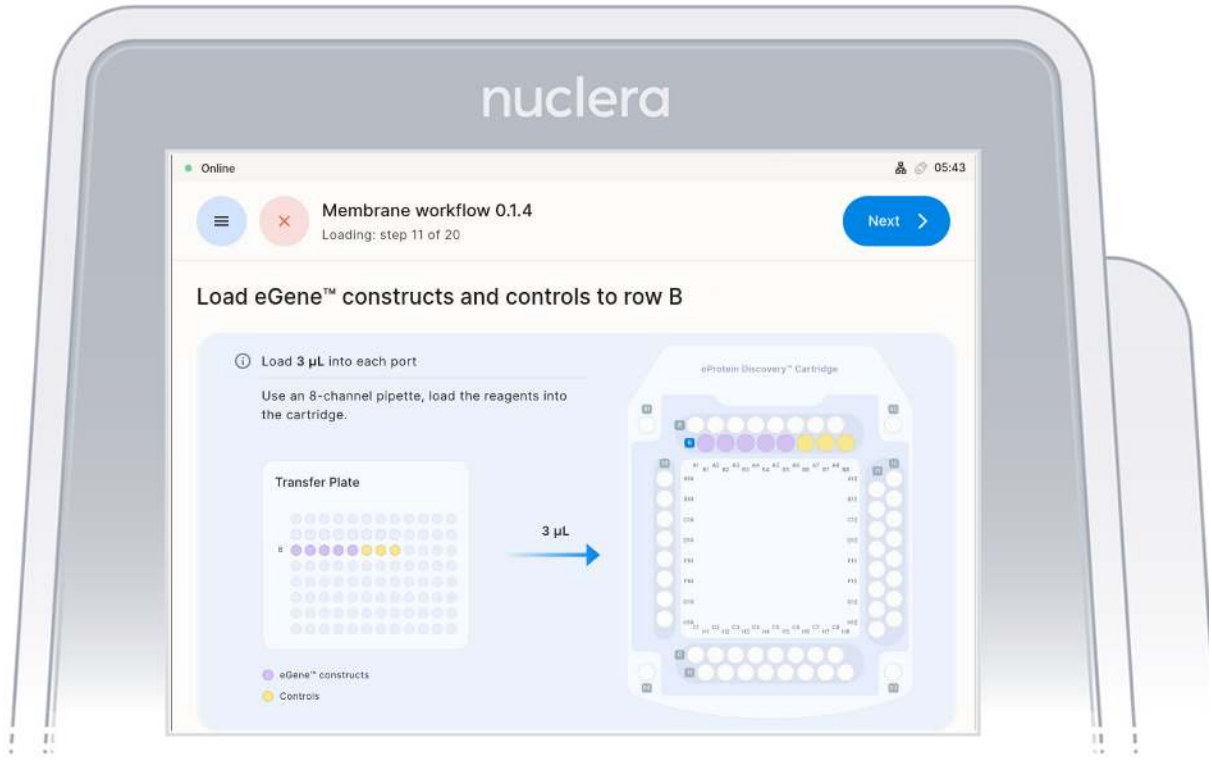
▹ Load x8 fresh p20 pipette tips and aspirate 3 µL of the reagents from the transfer plate wells B1-B8 into ports B1-B8 of the cartridge (Figure 21).

▹ Ensure the pipette tips are positioned just below the surface of the base fluid, away from the sides and bottom of the port.

▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.

▹ Eject the pipette tips into a waste container.

▹ Press the **[Next]** button on the screen.

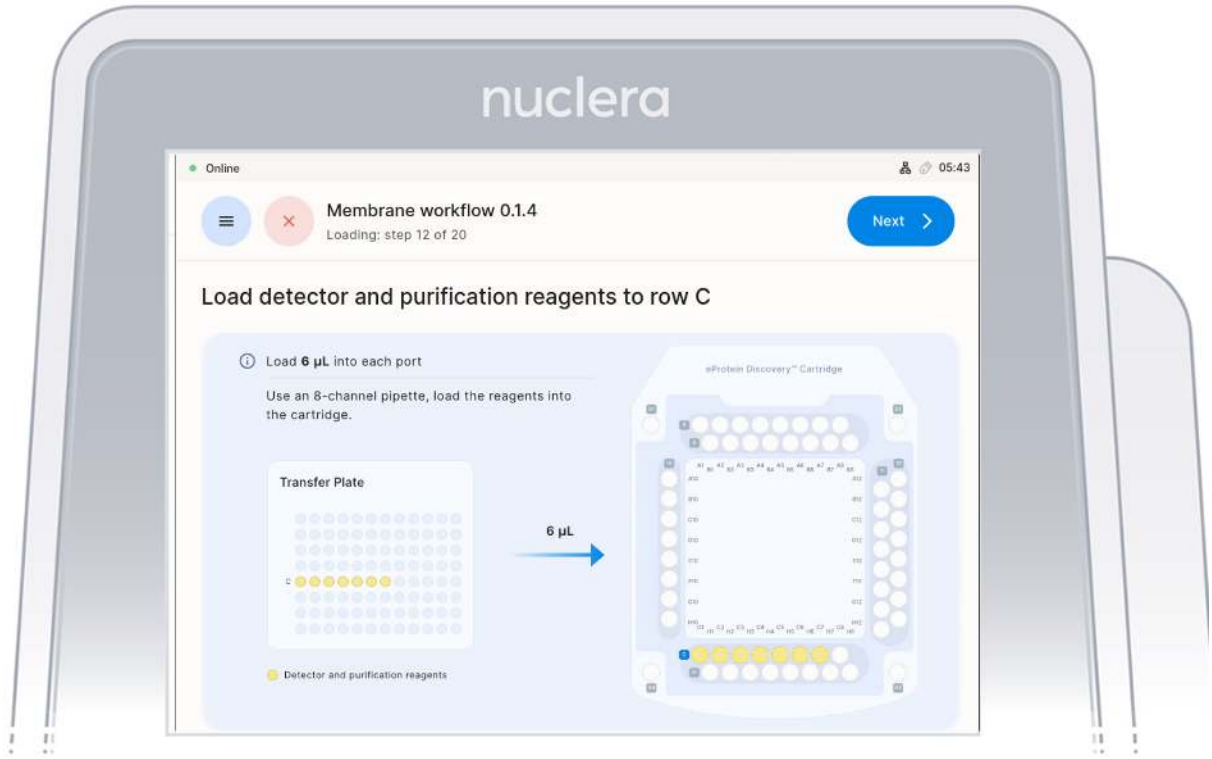



**3. Reagents - row C:**

▹ Load **7x** fresh p20 pipette tips and aspirate 6 µL of the reagents from the transfer plate wells C1-C7 into ports C1-C7 of the cartridge (Figure 20). **Do not use 8 tips as the purge of an empty tips might create air bubbles in the cartridge**

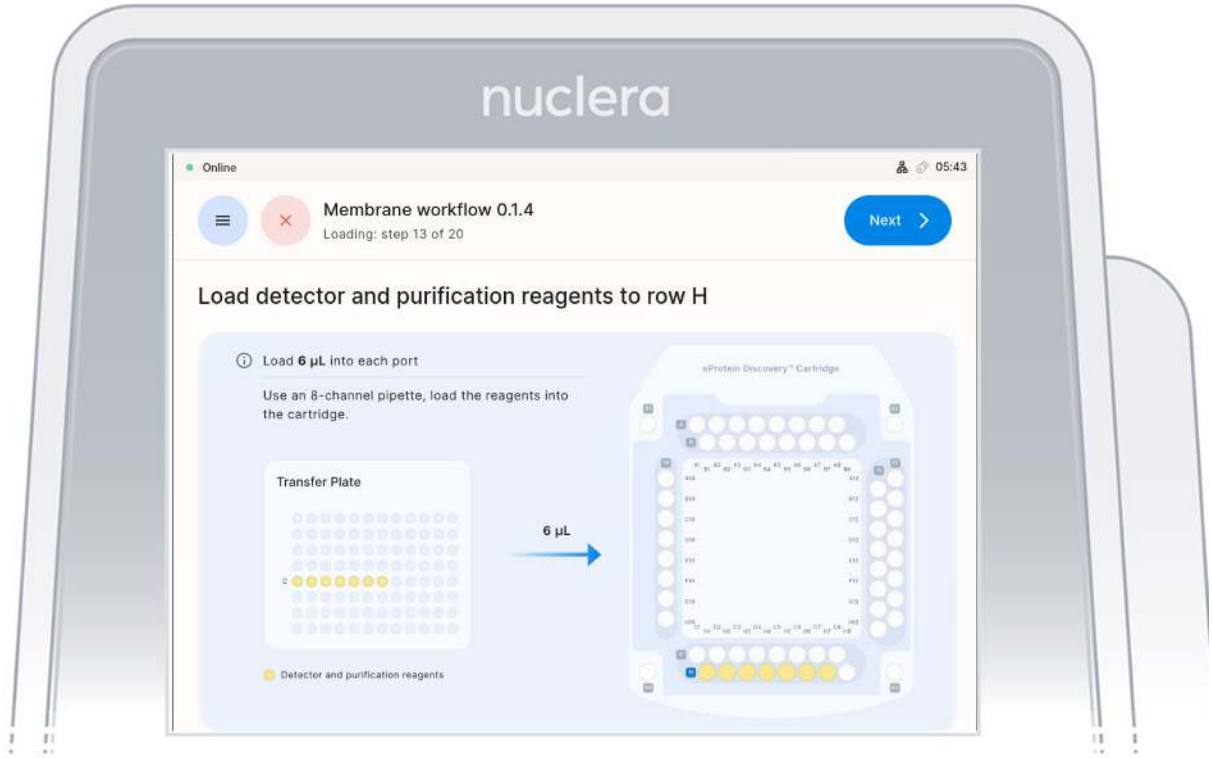
▹ Ensure the pipette tips are positioned just below the surface of the base fluid, away from the sides and bottom of the port.

▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.




**4. Reagents - row H:**

▹ Load **7x** fresh p20 pipette tips and aspirate 6 µL of the reagents from the transfer plate wells C1-C7 into ports H1-H7 of the cartridge (Figure 20). **Do not use 8 tips as the purge of an empty tips might create air bubbles in the cartridge**
▹ Ensure the pipette tips are positioned just below the surface of the base fluid, away from the sides and bottom of the port.
▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.

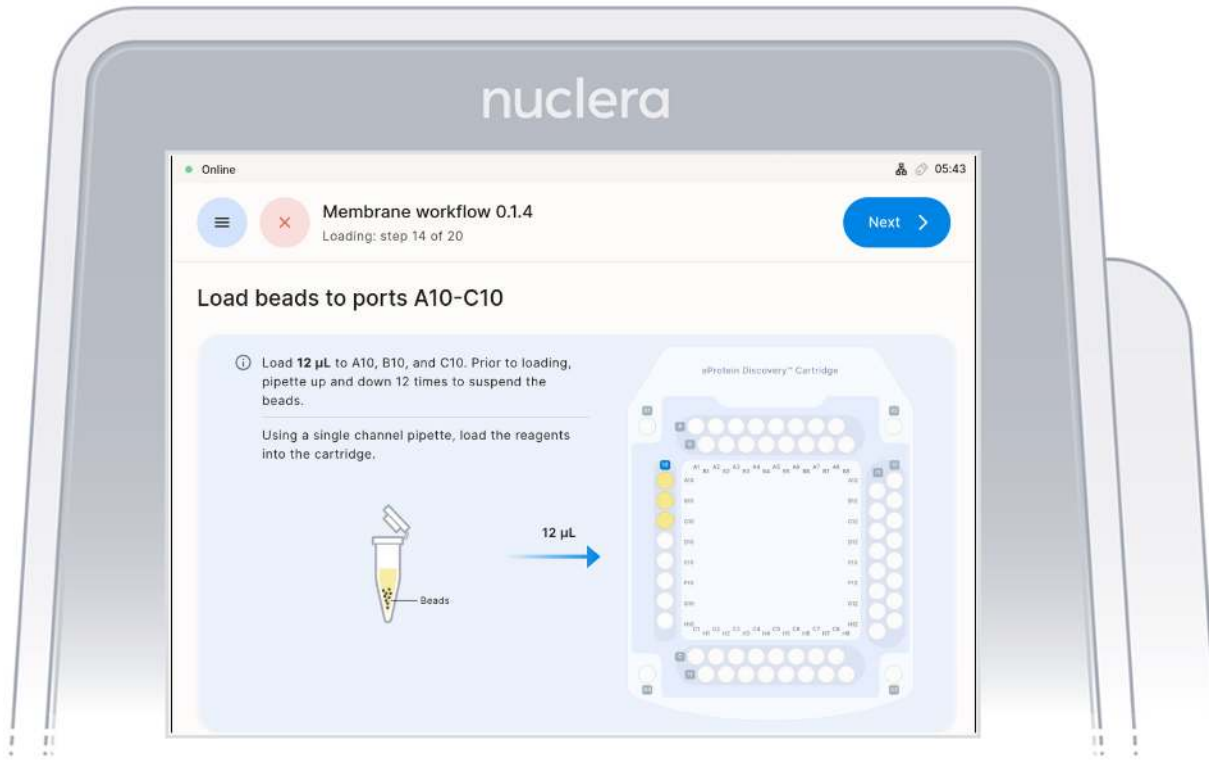



**5. Strep Purification Beads - ports A10-C10:**

▹ Using a single-channel P20 pipette, mix the first Strep Purification Beads tube by gently pipetting up and down 12 times, avoiding air bubbles. Immediately aspirate 12 µL and dispense into port A10 of the cartridge. Repeat the same process for the second and third tubes, dispensing into ports B10 and C10, respectively (Figure 22).

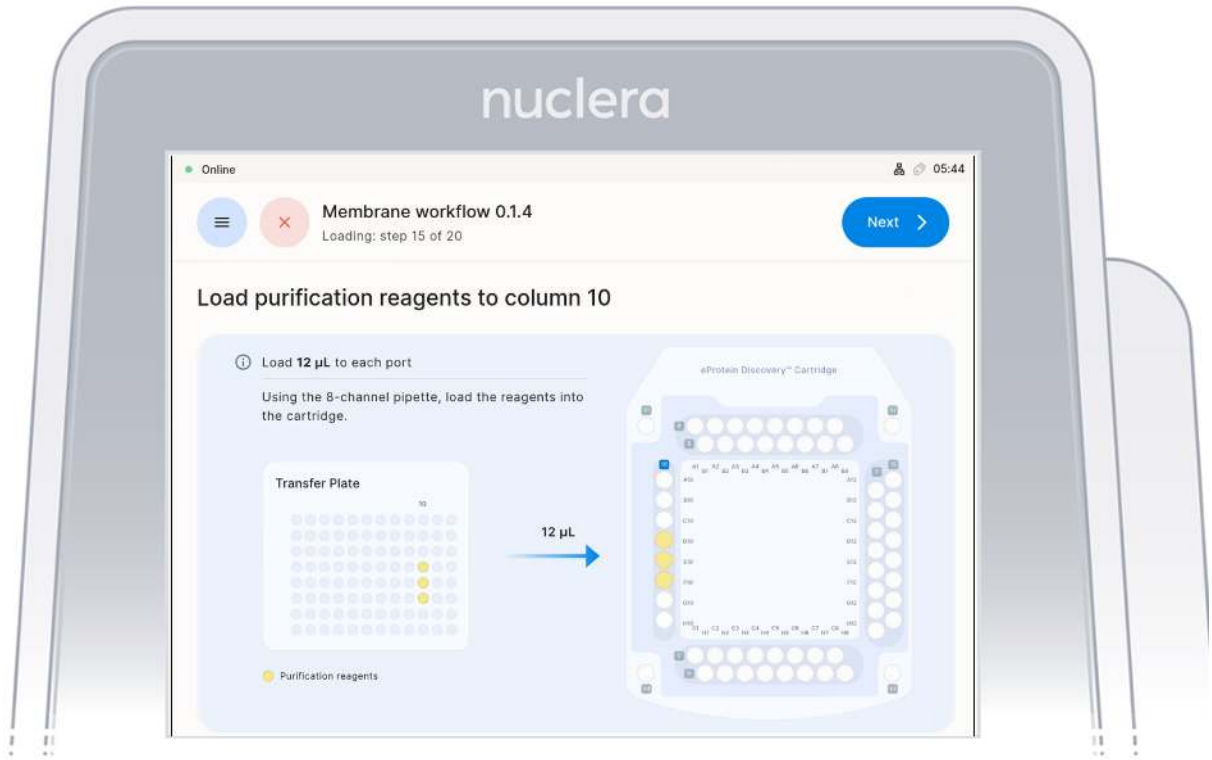
> **IMPORTANT NOTE**
>
> Mix, aspirate, and dispense each tube sequentially to prevent bead settling and ensure uniform loading in the cartridge.

▹ Ensure the pipette tip is positioned just below the surface of the base fluid, away from the sides and bottom of the port.
▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.
▹ Eject the pipette tip into a waste container.
▹ Press the **[Next]** button on the screen.




**6. Purification Reagents - ports D10-F10:**

▹ Aspirate 12 µL of purification reagents from D10-F10 wells of the trasfer plate and dispense it to column 10 on the cartridge (Figure 23).
▹ Ensure the pipette tips are positioned just below the surface of the base fluid, away from the sides and bottom of the port.
▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.

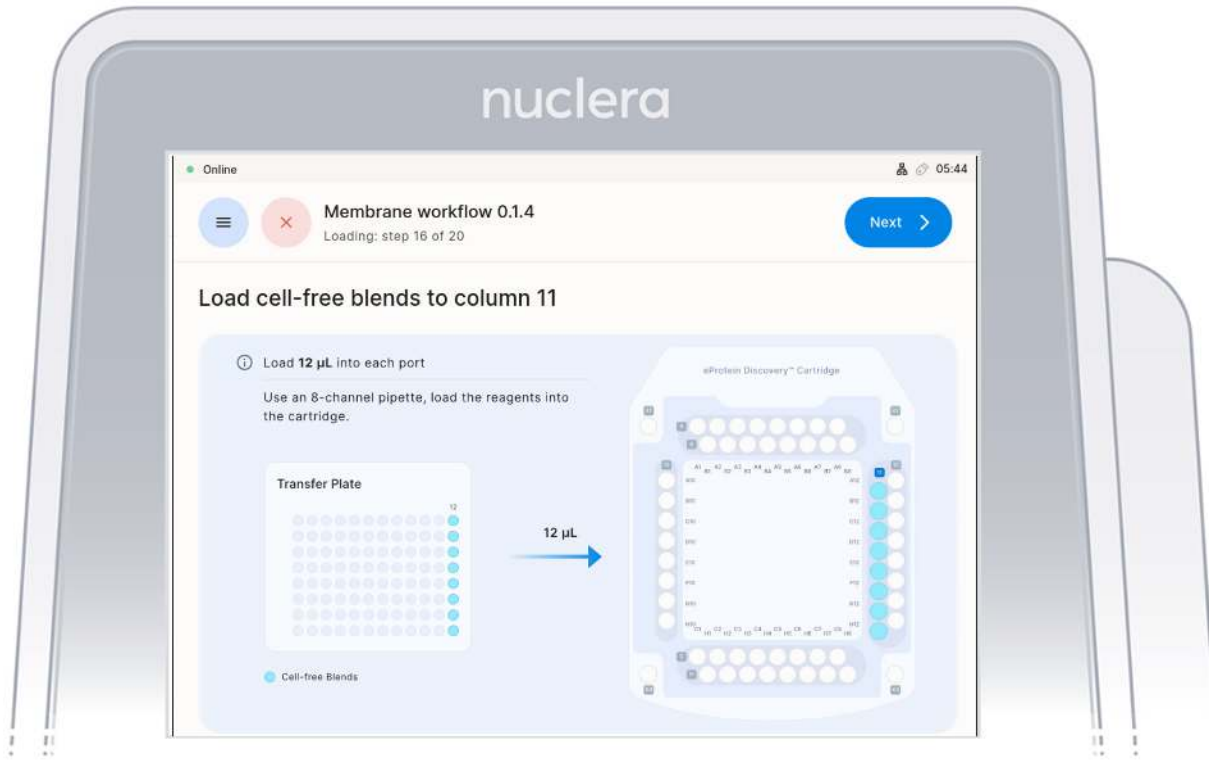



**7. Reagents - column 12:**

▹ Load 8x fresh p20 pipette tips and **mix the Cell-free Blends in the transfer plate in column 12 by gently pipetting up and down 12 times. **

> **IMPORTANT NOTE**
>
> Be careful not to introduce air bubbles in the ports.
> Aspirate 12 µL of the Cell-free Blends from the transfer plate wells

▹ Aspirate 12 µL of cell-free blend from the transfer plate wells and dispense it to column 11 on the cartridge (Figure 22).
▹ Ensure the pipette tips are positioned just below the surface of the base fluid, away from the sides and bottom of the port.
▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.




**8. Reagents - column 12:**

▹ Aspirate 3 µL of cell-free blend from the transfer plate wells and dispense it to column 12 on the cartridge (Figure 23).
▹ Ensure the pipette tips are positioned just below the surface of the base fluid, away from the sides and bottom of the port.
▹ Dispense slowly until the first stop of the pipette is reached. Do not insert the pipette tip directly into the base of port.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.


## Load reagents in the cartridge

1. Press the **[Next]** button to start the aspiration of the base fluid and the loading of the reagents on the cartridge (Figure 24).

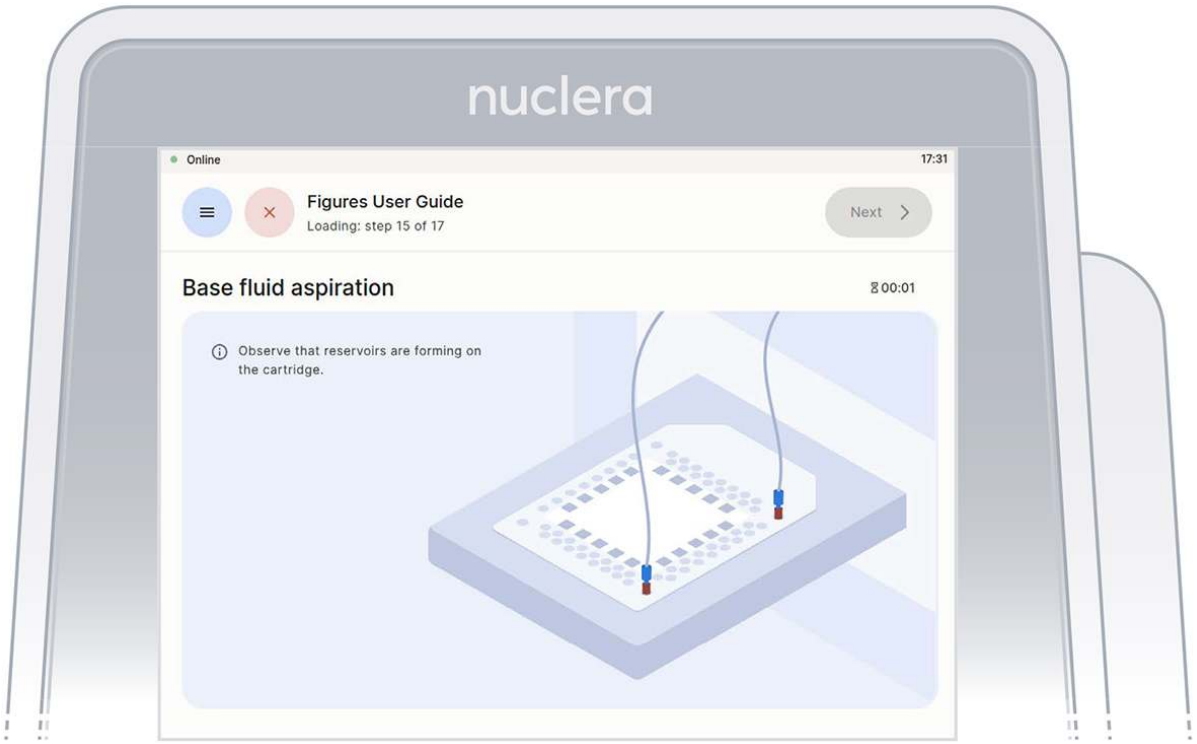



**Figure 24: Base fluid aspiration**

2. Disconnect the tubes from the cartridge and place them in the tube holder on the right hand side of the instrument. Press the **[Next]** button on the screen (Figure 25), and the drawer will close. Quality controls will be performed. The experiment will start.

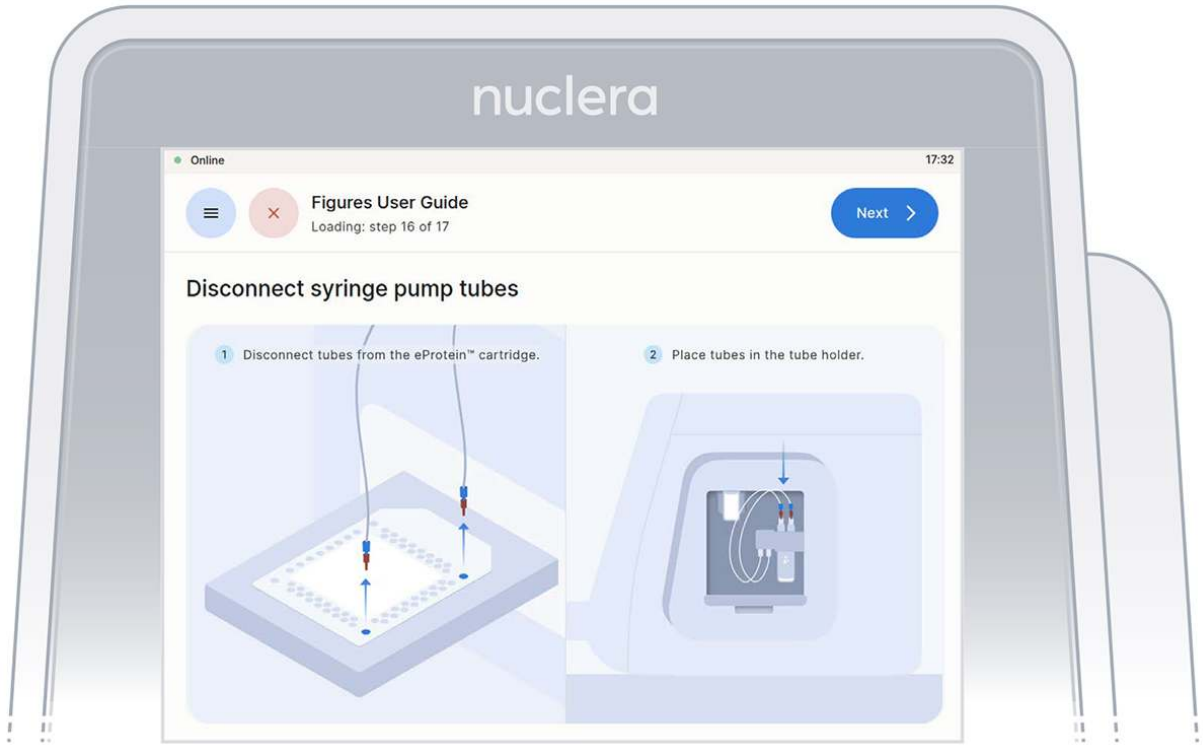



**Figure 25: Disconnect the tubes and place them on the tube holder**

# Analyze the results

### Instrument software results screen

After completion of the experiment, the results are shown on the instrument screen. The four best obtainable combinations of eGene and Cell-free Blend are displayed with the predicted in-tube scale-up yields (Figure 28). Further analysis can be carried out from the eProtein Discovery Cloud Software.

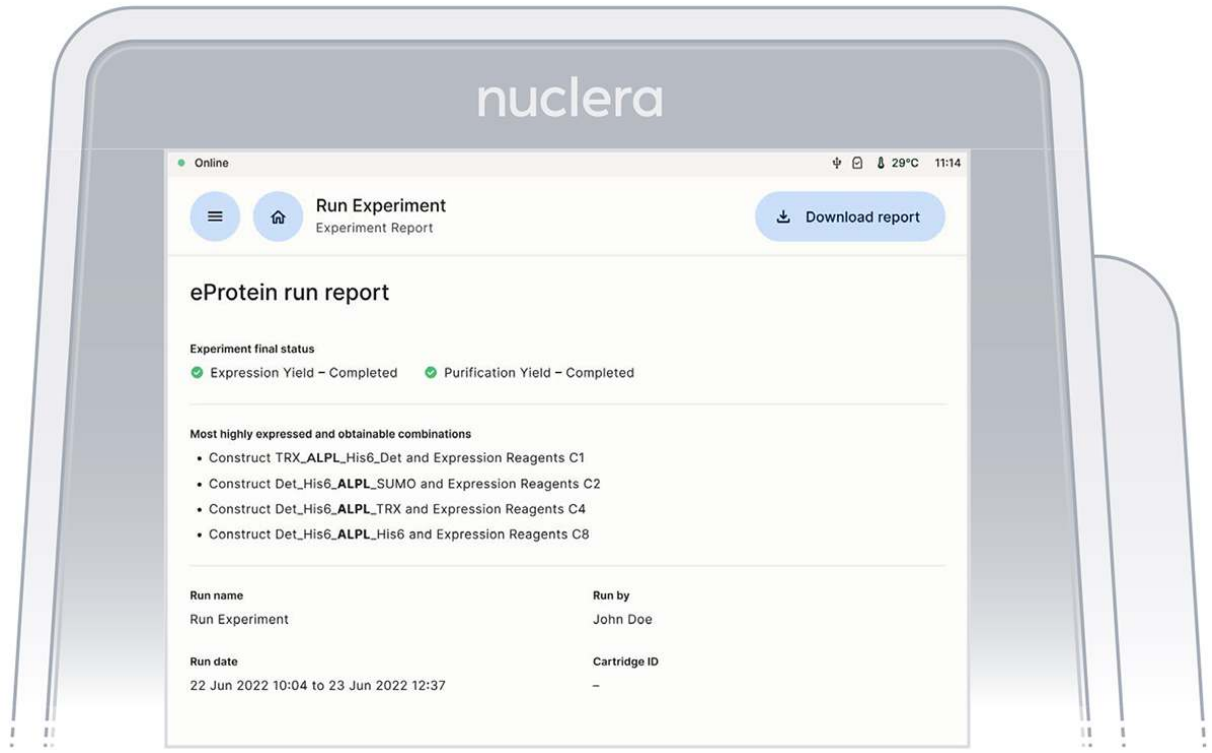



**Figure 28: Result screen from the Instrument Software**

## eProtein Discovery Cloud Software report

At the end of the experiment a report containing all the information about the experimental setup is transferred onto the eProtein Discovery Cloud Software. The upload takes about 15 minutes and during this time the **[Download Report]** button at the top right corner of the screen is grayed out.

**Note:** the instrument should not be switched off until the report is transferred and becomes available on the eProtein Discovery Cloud Software.

**The experiment report contains:**

▹ **Experiment video**

The video should be watched to ensure the correct operation of the instrument and cartridge during the experiment. Any questions or concerns regarding the operation of the droplets should be directed to the

Nuclera Technical Support team (techsupport@nuclera.com).

▹ **PDF report file**

The PDF report file is a summary of the experiment setup and the results, saved in the report folder with the name given to the experiment included in the file name

▹ **CSV report file**

The report file is a csv file saved in the report folder with the name given to the experiment included in the file name. The results for each one of the 88 expressed and purified protein conditions are listed in the csv file. It also contains the measured values for the controls, the expected range for the controls, and a PASS/FAIL score if the measured values are within the expected range.

▹ **Blue light images (TIFF images)**

Images acquired at the end of expressions and purification. These images can give the user information about the solubility of the protein.

▹ **Other files**

The folder contains additional files that can be used by the Nuclera Technical Support team for troubleshooting purposes

## Finishing the experiment

1. Remove the cartridge from the instrument drawer by lifting it as shown on the screen and place it in its original packaging. Press the **[Next]** button (Figure 29).

**Note:** there is no need to drain the base fluid out of the cartridge.


**Figure 29: Remove the cartridge from the instrument**

2. Remove the waste container from the holder, empty its content, and place it back on the instrument.
3. Remove the vial of base fluid and dispose of it with biohazard sharps waste container according to local waste disposal rules and regulations.

**Note:** Do not reuse consumed cartridges and dispose of any residual reagents, kits are intended as single use only.

4. Dispose the packaged used cartridge in a biohazard sharps container, according to local waste disposal rules and regulations.
5. The experiment report is available for download from the eProtein Discovery Cloud Software.
6. Power down the instrument after use by pressing the [Power off] button (Figure 30)

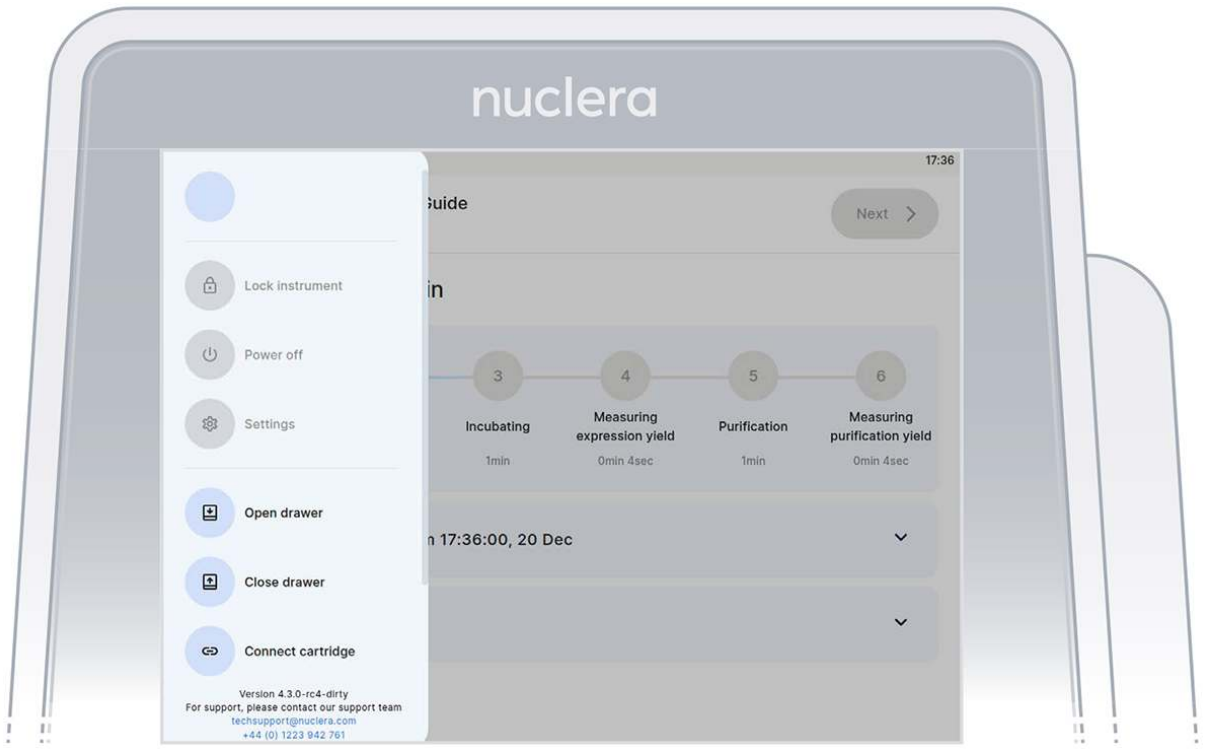



**Figure 30: Remove the cartridge from the instrument**

---

**Nuclera Technical Support:**

UK Phone: +44 1223 942 761

US Phone: +1 508-306-1297

Email: techsupport@nuclera.com

**Offices:**

Nuclera UK (HQ):

One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:

1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

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# eProtein Discovery™ System - Standalone

## eProtein Discovery system

📄 Download Page as PDF

### General information

eProtein Discovery™ is the only end-to-end protein screening system that accelerates construct design, expression, solubility characterization and purification of target proteins in drug discovery programs. Accelerating the journey to your protein.

▹Rapid protein screening accelerates scientific progress by allowing researchers to quickly determine which proteins and their variants are optimal for achieving soluble, high yield proteins
▹Simultaneously screen multiple constructs and protein synthesis reagents for soluble expression, and then scale up to micrograms of recombinant protein off cartridge to test in your applications.
▹Explore multiple DNA constructs, including solubility tags, polymorphisms and isoforms on the same cartridge to expand your range of accessible proteins.

### Four system components. One complete protein solution.

**Instrument:** With protein prototyping, you can draw a straight line from

theory to reality, allowing you to test hypotheses more efficiently and focus on promising targets. The eProtein Discovery™ instrument puts rapid protein prototyping on your benchtop.
Designed for all levels of scientist, it streamlines your workflow and grants you the ability to identify optimal DNA constructs, test expression feasibility earlier, and pursue targets with confidence. Fail fast, succeed faster!

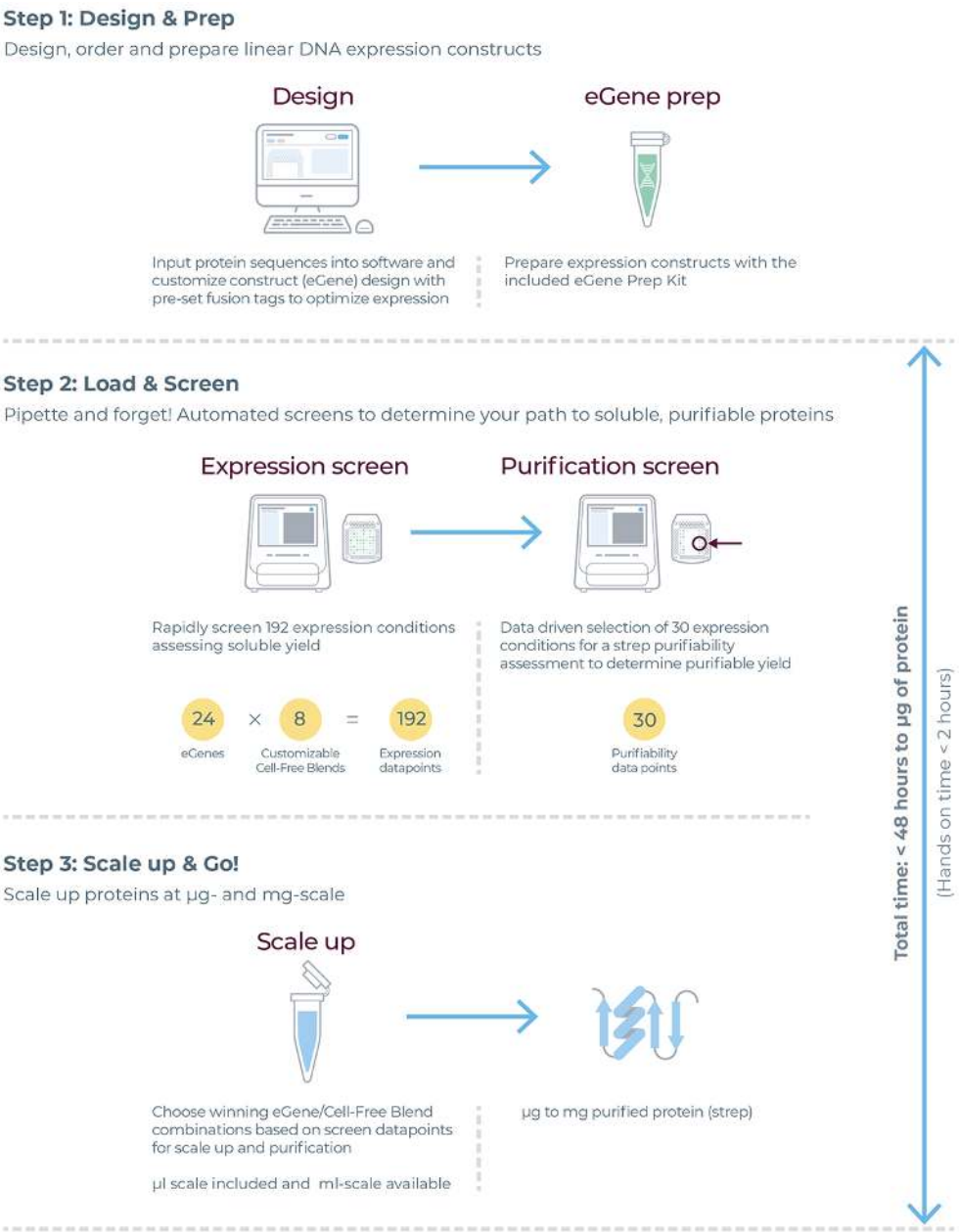
**Software:** eProtein Discovery™ software simplifies a complex multivariate experimental design. The software sets up and simultaneously tracks 192 different combinations of DNA sequences, flank pairs and expression reagent reactions performed on eProtein Discovery™ system. AI performs highly rigorous QA checks during an experiment to ensure data quality and consistency.

**Cartridge:** Powered by digital microfluidic technology, software controlled digital signals guide the movement of droplets on the eProtein Discovery™ Cartridge surface to enable splitting, dispensing and merging of biological reagents. Pipette DNA, cell free expression reagents and purification solutions on the Cartridge and the technology will orchestrate the rest.
Gain precise control of your eGene™ constructs and reagents to screen and discover optimal expressing conditions within 24 hours, accelerating target selection. A simple set-up allows anyone to run the system with minimal training.

**Reagents:** The reagents within the eProtein Discovery™ system allow you to optimize protein obtainability by characterizing and purifying different combinations of DNA constructs and expression conditions. Our system will screen 192 different combinations in 24 hours for you to select the optimal conditions to scale up and get protein.
Our eProtein Discovery™ software will guide you in creating the panel of DNA constructs and reagents to power your experiment. Our complete reagent package includes design and ordering of DNA, simplifying your

workflow.

# eProtein Discovery Workflow



# eProtein Discovery product contents

### Equipment

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| eProtein Discovery Instrument | 1 unit | Room Temperature | N1001 |  |

---

### Cartridge Kit NC3006 - Consumables

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| eProtein Discovery Cartridge | 1 unit | Room Temperature | NC3006 |  |
| eProtein Discovery Cartridge Cover | 1 unit | Room Temperature | NC3012 | |

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| Base Fluid | 1 unit | Room Temperature | NC3007 |  |

---

### Cartridge Reagent Kit +4°C reagent - NC3010-2

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| Strep Beads | 200 µL | +4°C | NC3010-2 |  |

---

**Cartridge Reagent Kit -80°C reagents - NC3010-1 (yellow stripe on label)**

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| Cell Free Core Reagent | 160 µL | -80°C | SC3-01 | |
| Blank Buffer | 150 µL | -80°C | SC3-02 | |
| Detector Protein* | 75 µL | -80°C | SC3-03 | |
| Universal Control* | 20 µL | -80°C | SC3-04 | |
| Complementation Control* | 20 µL | -80°C | SC3-05 | |
| Expression Control* | 20 µL | -80°C | SC3-06 |  |
| Full Workflow Control* | 20 µL | -80°C | SC3-07 | |
| Wash Buffer* | 800 µL | -80°C | SC3-08 | |
| Elution Buffer* | 50 µL | -80°C | SC3-09 | |
| AdditiveBuffer* | 50 µL | -80°C | SC3-10 | |
| PDI/GSSG Mix* | 50 µL | -80°C | SC3-11 | |

| Description | Quantity | Storage Temperature | Product Code | |
|---|---|---|---|---|
| TRXB1* | 50 µL | -80°C | SC3-12 | |
| DNAk Mix* | 50 µL | -80°C | SC3-13 | |
| Zinc Chloride | 50 µL | -80°C | SC3-14 | |
| Calcium Chloride | 50 µL | -80°C | SC3-15 | |
| Manganese Chloride | 50 µL | -80°C | SC3-16 | |
| Cofactor Mix* | 50 µL | -80°C | SC3-17 | |
| GSSG* | 50 µL | -80°C | SC3-18 | |
| 3C protease* | 50 µL | -80°C | SC3-19 | |

Reagents must be used before the expiration date indicated on the kit box.

* Single use reagent that cannot be freeze/thawed multiple times.

### User supplied reagents

- 5 nM eGene constructs (DNA), stored at -80°C, generated using the Nuclera eGene Prep kit NC3008 or NC3009

### User supplied equipment

- Magnetic particle separator (compatible with 1.5 mL microcentrifuge tubes)

- Vortexer
- Microcentrifuge
- 1.5 mL microcentrifuge tubes
- 2-20 µL 8-channel pipette
- 2-20 µL single-channel pipette
- 200 µL compatible tips

## Protein Variant Creation

The purpose of this guide is to describe a guided approach for designing protein variants, mutants, and truncated sequences.

Support users in generating variants of their protein to test on the eProtein Discovery platform and increase their chances to get quickly soluble, functional protein to use for downstream applications in their project.

**Summary - A stepwise guided method for variant creation**

▹ **Step 1 - Identify Relevant UniProt ID** Use sequence alignment (POI sequence) or direct UniProtID input to identify the starting protein sequence and/or several close protein family members - for example isoforms and splice variants. Annotate each starting sequence with all required metadata.

▹ **Step 2 - Select Candidates** Filter isoforms, align them and flag functional or structural domains of interest.

▹ **Step 3 - Rule-based Sequence Editing I** Apply simple rule based editing for each input Candidate. Depending on the domains present, each input Candidate sequence should generate several “virtual” constructs. Remove signal peptides and propeptides, it is also often beneficial to remove transmembrane domains (TMD).

▹ **Step 4 - Rule Based Sequence Editing II - Terminal truncations** Apply simple rule based editing for each input Candidate. Consider modifications around functional domains of interest, for example removing disordered or unnecessary domains. eProtein Discovery™ System User Guide 9

▹ **Step 5 - Check for other known stable domains (NMR, X-Ray)** Identify other important regions and create relevant variants.

▹ **Step 6 - Compile final list of variant Candidates for a POI**

A general guideline for manually designing gene fragments compatible with the eGene Prep Kit is available upon request, provided a Non-Disclosure Agreement (NDA) is in place. If you are unable to use the eProtein Discovery Cloud Software to design gene fragments and need further assistance with constructing fragments compatible with the eGene Prep Kit, please contact Technical Support. (techsupport@nuclera.com)

**Details - A stepwise guided method for variant creation**

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| 1 | Identify Relevant Uniprot ID | Sequence or Uniprot ID | Annotated Uniprot sequences | • Identify relevant isoform, canonical isoforms, orthologs, align.<br>• Identify critical domains, |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | Uniprot, Expasy (Structural, functional, etc.) |
| 2 | Select Initial Candidates | Annotated Uniprot sequences | Isoforms and important domains flagged | • Identify relevant isoforms, canonical iso forms, orthologs, align<br>• Identify critical domains, Uniprot, Expasy (Structural, functional, etc.) |
| 3 | Combine starting list | Seqs from steps 1 and 2 | List of input Candidates | Combine lists 1 and 2 |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| 4 | Rule-based SequenceEditing I - identify domains of interest | List of input Candidates | List of Child Candidates 1 Edited sequences named appropriately - rules applied see operations.<br><br>A Child Candidate is a sequence derived from an Initial Candidate by applying Rule-based Editing - Step 4 | • If present remove signal peptide from N-terminus If present remove pro-peptide from N-terminus or C-terminus<br>• If 1 TMD present remove domain * If >1 TMD present discard Candidate sequence<br>• If N-term TMD truncate after TMD (e.g. aa directly after TMD)<br>• If C-term TMD truncate before TMD (e.g. aa directly before |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | TMD) |
| 5 | Rule Based Sequence Editing II - truncations | List of input candidates (Step 3)<br>+<br>List of Child Candidates (step 4) | | • Consider N-terminal truncation to leave the domain of interest. Users may add up to 10 aa upstream.<br>• Consider C-terminal truncation to leave the domain of interest. Users may add up to 10 aa downstream.<br>• Consider both N- and C-terminal truncations to leave the domain of |

| Step | Title | Input | Output | Operations |
|---|---|---|---|---|
| | | | | interest. Users may add up to 10 aa upstream or downstream.<br>• Consider truncating to remove disordered regions around functional domain of interest<br>• If available use structure information (X-ray, NMR, AlphaFold) to guide truncation sites |
| 6 | Compile final Screening Candidates | List of input candidates (Step 3) | | • Consider your downstream needs (e.g. |

| Step | Title | Input | Output | Operations |
| --- | --- | --- | --- | --- |
| | | + List of Child Candidates (step 4) + List of Child Candidates (step 5) | | activity assay, binding assay, structural investigation)<br>• Consider your cartridge format (e.g. a FlexiVariant™ or Solubility tag screen<br>• Consider your cartridge budget (e.g. do you plan on running one or more cartridges within this campaign)<br>• Consider Alphafold scoring results |

# Additive Selection Guide

Depending on the protein of interest, the presence of additives may be

needed to optimize the expression. We recommend that you review the feature, function and binder of each protein to help guide the selection of additives. These information can be found in Uniprot.

The decision tree in Figure 1 illustrates the Additive options based on protein requirements.

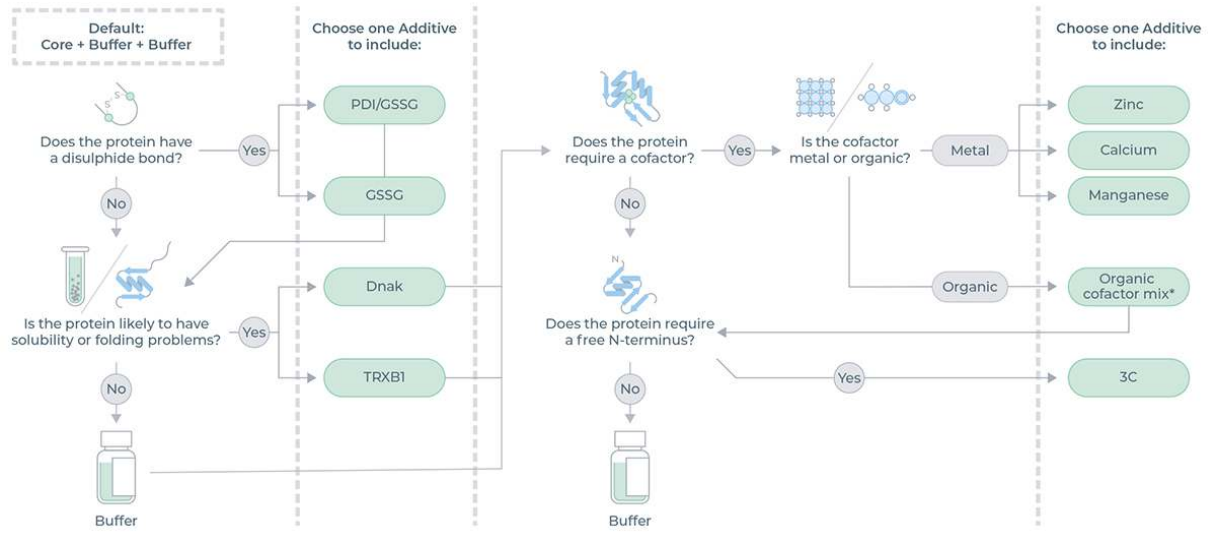



**Figure 1: Decision tree which illustrates the Additive options based on protein requirements.**

*Mix of NAD, acetyl-CoA, FAD, SAM and PLP.

The additives supplied in the Cartridge Reagent Kit NC3010 and their descriptions are listed in Table 1.

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| Additive buffer | HEPES buffer pH 7.5 and surfactant | CFPS reaction buffer, dilution normalization |

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| PDI + GSSG Mix | Protein disulfide isomerase and oxidized glutathione | Chaperone and redox modification to oxidizing environment to support disulfide bond formation |
| TrxB1 | Thioredoxin reductase | Protects proteins from oxidative aggregation and inactivation and acts as a reductase in redox regulation |
| DnaK Mix | Chaperone | DnaK mix Chaperone mix to support folding and prevent aggregation |
| Zinc chloride | Zinc chloride solution | Cofactor that can be required for folding, stability, or activity |
| Calcium chloride | Calcium chloride solution | Cofactor that can be required for compaction, folding, stabilization, or activity |
| Manganese chloride | Manganese chloride solution | Cofactor for metalloenzymes for structure and activity |
| Cofactor Mix | Mix of NAD, acetyl CoA, FAD, SAM, and PLP | Cofactors that assist in folding, stability and activity |

| Additive | Additive Description | Additive Characteristics |
|---|---|---|
| GSSG | Oxidized glutathione | Redox modification to oxidizing environment |
| 3C protease | 3C protease solution | Protease to cleave off the N-terminal solubility tag at the specific aminoacid sequence (LEVLFQ/GP) |

**Table 1: List of Additives supplied in the Cartridge Reagent Kit NC3010.**

# eProtein Discovery Software

### Intended Use

The eProtein Discovery Software supports the user in the design and execution of combinatorial protein expression experiments on the eProtein Discovery platform.

### Software Updates

▹ Software updates can be manually installed by connecting temporarily the instrument to the internet. Note: this applies if the instrument is able to connect to the eProtein Discovery Cloud Software.

# Design an experiment

To design an experiment, use the Nuclera_eProtein_Discovery_Standalone_template (Excel file) available from the Nuclera Cloud Software, or on request by contacting the Nuclera Technical Support team (techsupport@nuclera.com).

> **NOTE**
>
> The Nuclera_eProtein_Discovery_Standalone_template file is compatible with Microsoft Excel and it is not compatible with Google Sheet.

The excel template includes multiple worksheets. The worksheets provide step-by-step guide in designing the experiment and analyze data. Read the Template Guidance sheet before proceeding with experiment design.

Open the Excel file, enable editing, and save it under the name of your choice, for example the name or the date of the experiment.

**"Template Guidance" sheet**

This sheet contains a general introduction and guidance on how to use it. The first steps are to enable editing of the document and to save the file.

**"1. Enter Experiment Details" sheet**

In this sheet you will design your experiment by adding the combinations of proteins of interest (POI), solubility tags and additives selected for the experiment. Enter the requested information in the cells highlighted in yellow (Figure 2)

1. Select the format of your experiment (3 proteins x 8 solubility tags, or 4x6 or 6x4 or 24x1)
2. Enter the name of the proteins of interest
3. Enter the molecular weights (kDa) for each protein of interest
4. Select from the drop down menu the solubility tags chosen for the experiment
5. Select from the drop down menus the two additives required for your experiment

**NOTE**

The “Additive selection guide” section can help to select the right additives.
The eProtein Discovery™ system also provides the flexibility to incorporate custom additives into expression and purification workflows, enabling users to tailor conditions for unique protein targets. To ensure optimal performance and minimize risks, please consult the Compatibility List (https://info.nuclera.com/manual-custom-additives-chemical-compatibility-list.html), which provides detailed guidelines on compatible additives and their maximum allowed concentration. This resource serves as a valuable reference to help you achieve optimal results when working with custom additives. Refer to this before experimenting with custom additives or contact Technical Support if you require more guidance.

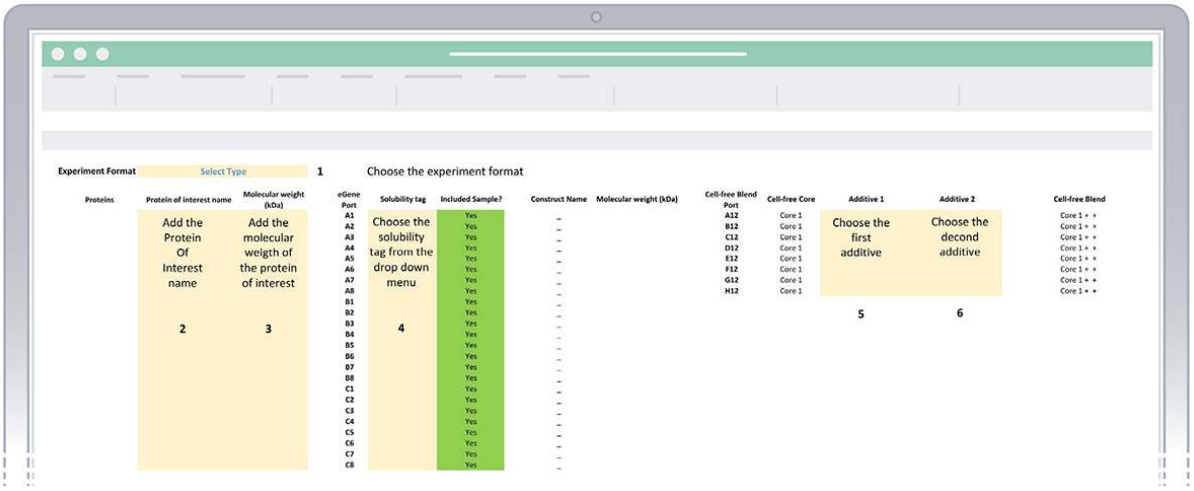
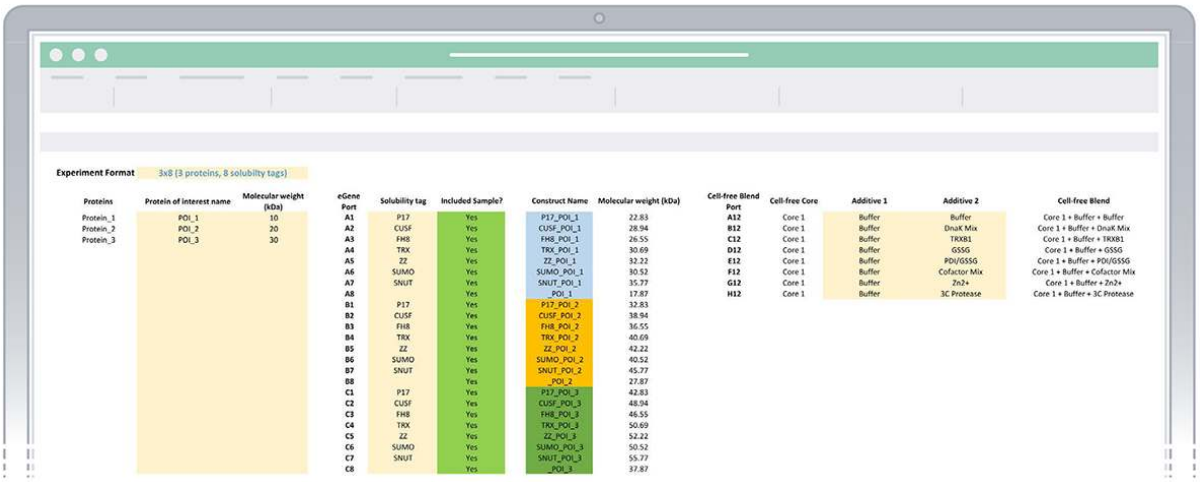



**Figure 2: Sheet “1. Enter experiment details” of the eProtein Discovery Standalone template.**

6. Once the five steps outlined above are completed, the white columns for Construct, Molecular weight (KDa), Protein, and Cell-free Blend will be automatically populated with more information (Figure 3).



Experiment Format: 3x8 (3 proteins, 8 solubilty tags)

| Proteins | Protein of interest name | Molecular weight (kDa) |
|---|---|---|
| Protein_1 | POI_1 | 10 |
| Protein_2 | POI_2 | 20 |
| Protein_3 | POI_3 | 30 |

| eGene Port | Solubility tag | Included Sample? | Construct Name | Molecular weight (kDa) |
|---|---|---|---|---|
| A1 | P17 | Yes | P17_POI_1 | 22.83 |
| A2 | CUSF | Yes | CUSF_POI_1 | 28.94 |
| A3 | FH8 | Yes | FH8_POI_1 | 26.55 |
| A4 | TRX | Yes | TRX_POI_1 | 30.69 |
| A5 | ZZ | Yes | ZZ_POI_1 | 32.22 |
| A6 | SUMO | Yes | SUMO_POI_1 | 30.52 |
| A7 | SNUT | Yes | SNUT_POI_1 | 35.77 |
| A8 | | Yes | _POI_1 | 17.87 |
| B1 | P17 | Yes | P17_POI_2 | 32.83 |
| B2 | CUSF | Yes | CUSF_POI_2 | 38.94 |
| B3 | FH8 | Yes | FH8_POI_2 | 36.55 |
| B4 | TRX | Yes | TRX_POI_2 | 40.69 |
| B5 | ZZ | Yes | ZZ_POI_2 | 42.22 |
| B6 | SUMO | Yes | SUMO_POI_2 | 40.52 |
| B7 | SNUT | Yes | SNUT_POI_2 | 45.77 |
| B8 | | Yes | _POI_2 | 27.87 |
| C1 | P17 | Yes | P17_POI_3 | 42.83 |
| C2 | CUSF | Yes | CUSF_POI_3 | 48.94 |
| C3 | FH8 | Yes | FH8_POI_3 | 46.55 |
| C4 | TRX | Yes | TRX_POI_3 | 50.69 |
| C5 | ZZ | Yes | ZZ_POI_3 | 52.22 |
| C6 | SUMO | Yes | SUMO_POI_3 | 50.52 |
| C7 | SNUT | Yes | SNUT_POI_3 | 55.77 |
| C8 | | Yes | _POI_3 | 37.87 |

| Cell-free Blend Port | Cell-free Core | Additive 1 | Additive 2 | Cell-free Blend |
|---|---|---|---|---|
| A12 | Core 1 | Buffer | Buffer | Core 1 + Buffer + Buffer |
| B12 | Core 1 | Buffer | DnaK Mix | Core 1 + Buffer + DnaK Mix |
| C12 | Core 1 | Buffer | TRXB1 | Core 1 + Buffer + TRXB1 |
| D12 | Core 1 | Buffer | GSSG | Core 1 + Buffer + GSSG |
| E12 | Core 1 | Buffer | PDI/GSSG | Core 1 + Buffer + PDI/GSSG |
| F12 | Core 1 | Buffer | Cofactor Mix | Core 1 + Buffer + Cofactor Mix |
| G12 | Core 1 | Buffer | Zn2+ | Core 1 + Buffer + Zn2+ |
| H12 | Core 1 | Buffer | 3C Protease | Core 1 + Buffer + 3C Protease |

**Figure 3: Example of sheet “1. Enter experiment details” with experiment details entered.**

## "2. Print plate map" sheet

This sheet is the printable version of the experiment design. It is recommended to print this sheet and take it to the lab as a guide for loading reagents onto the transfer plate.

7. On the same sheet, below the table, you can find the transfer plate design (Figure 4). This design will help you determine where to load reagents on the transfer plate.

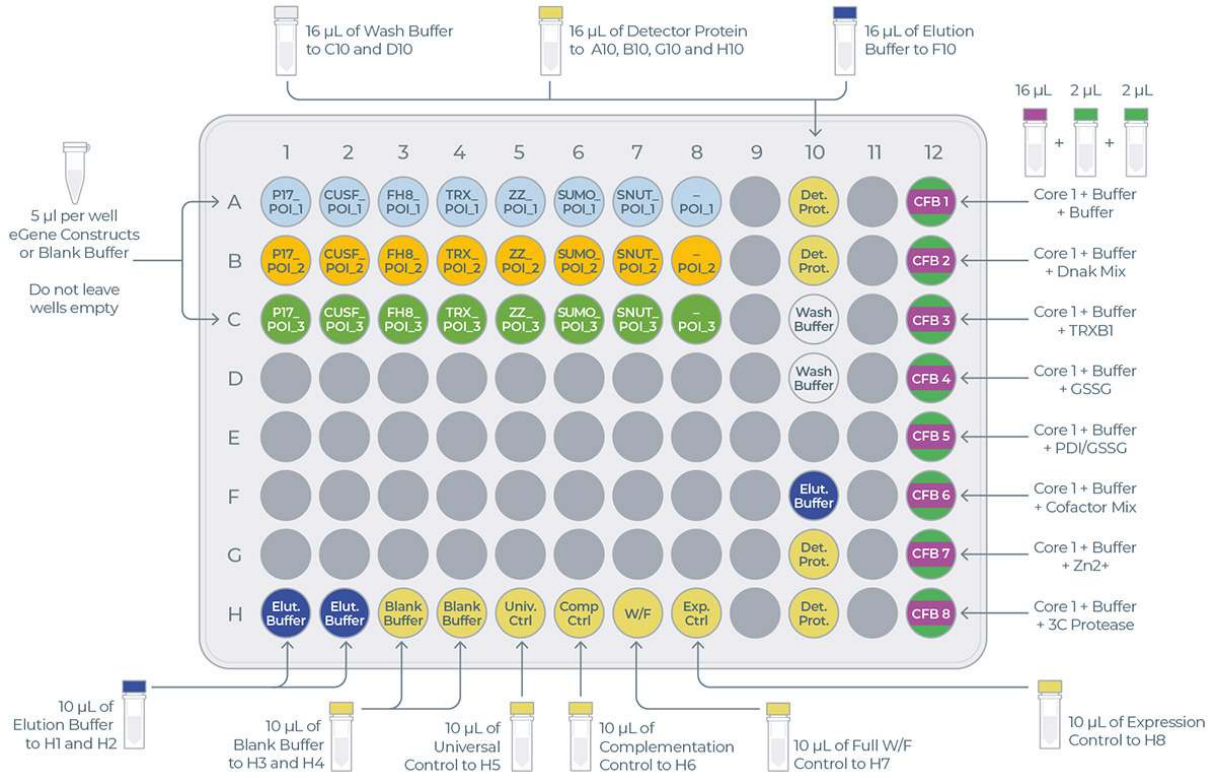



**Figure 4: Example of transfer plate design**

# Preparation of the eProtein Discovery reagents

The preparation of the reagents takes about 1 hour.

### Connect the vial of base fluid to the pump module

▹ In anticipation of starting a new experiment, take a fresh vial of base fluid, open it, and connect it to the left holder on the eProtein Discovery instrument pump module (Figures 5 and 6).

> **IMPORTANT NOTE**
>
> It is important to equilibrate the base fluid with the lab atmosphere prior to use. This is to prevent outgassing of the base fluid during the run, as air bubbles can interfere with the droplet movement. **We**

**recommend attaching the base fluid to the instrument the day before you will perform the run. An acceptable alternative is to incubate the uncapped base fluid at 30°C / 86°F for 1 hour**.

▹ Connect an empty waste vial to the right holder of the pump module (Figures 6 and 7).

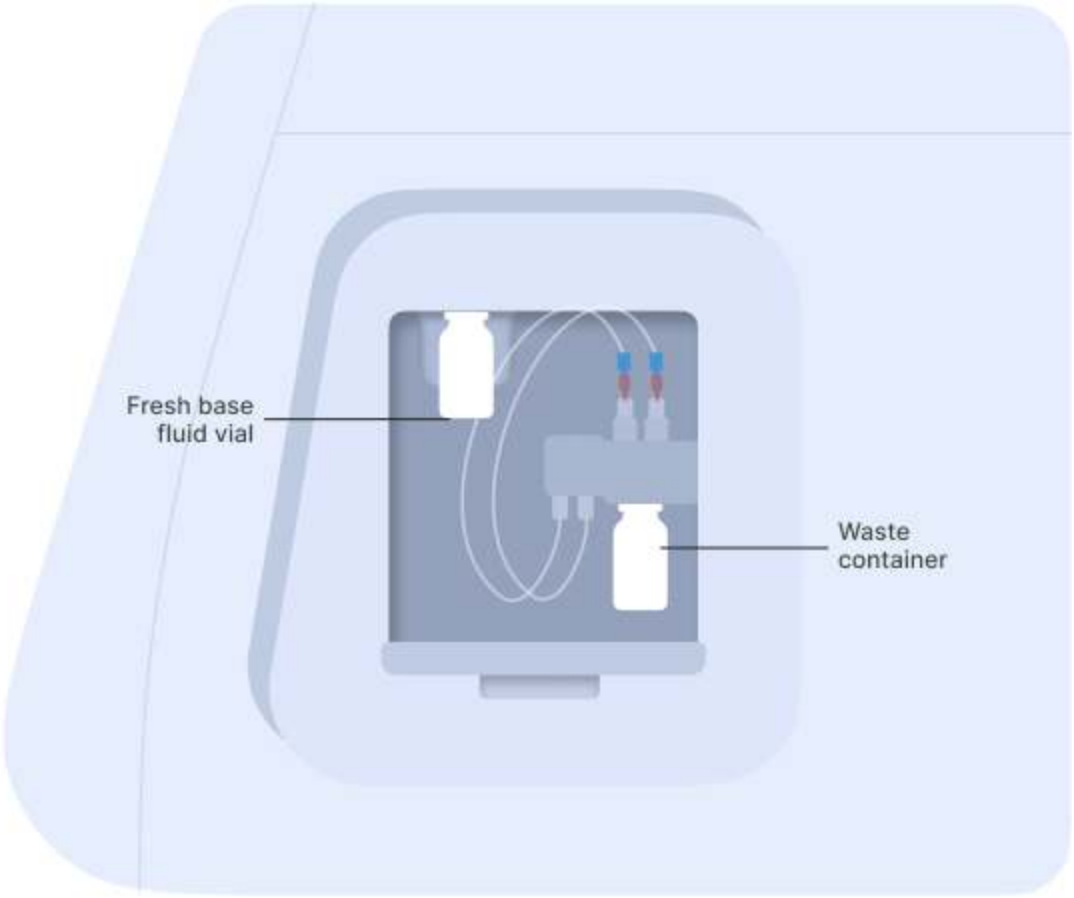



**Figure 5: Vial of base fluid and the waste container connected to the pump as shown on the screen**

[1]  [2]

**Figure 6: Connection of the empty waste container [1] and the vial of base fluid [2] to the pump**

## Prepare the transfer plate

After checking that the base fluid has been attached overnight to the instrument, take all the reagents out of the freezer.

The eProtein Discovery reagents need to be prepared and loaded onto a 96 well transfer plate following the layout and volumes in Figure 7 and Table 2.

> **NOTE**
>
> it is critical to follow this layout exactly because it determines how the reagents are dispensed in the eProtein Discovery cartridge.

**IMPORTANT NOTE**

It is critical not to leave any port empty. If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit, **not with water.**

**Tip:** Empty ports can be used for duplicates.

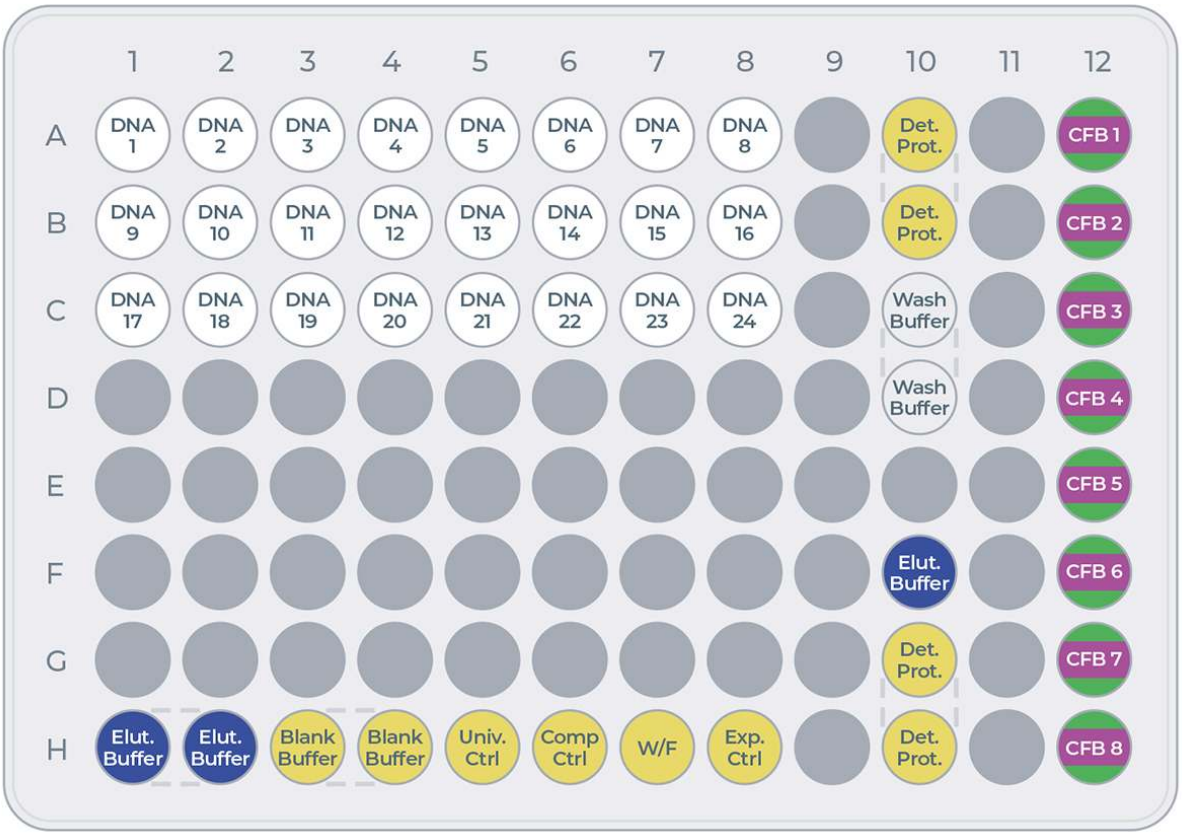



**Figure 7: Transfer plate layout**

| Reagent | Volume (µL) |
|---|---|
| eGene construct | 5 |

| Reagent | Volume (µL) |
|---|---|
| Controls: Blank Buffer, Universal Control (Univ. Ctrl), Complementation control (Comp. Ctrl), Full Workflow Control (W/F), Expression Control (Exp Ctrl) | 10 |
| Cell-free Blend (CFB): Cell-free Core Reagent + Additive 1 + Additive 2 | 20 (16+2+2) |
| Wash Buffer (Wash Buffer) | 16 |
| Elution Buffer (Elut. Buffer) | 10 µL in H1 & H2, 16 µL in F10 |
| Detector Protein (Det. Prot.) | 16 |

**Table 2: Reagents and volumes to load on the transfer plate.**

**1. Take the Strep Beads from the fridge and the Cartridge Kit reagents (box with the yellow stripe on the label) from the -80°C freezer.**


**2. Place an empty 96-well transfer plate on ice.**

**Note:** The transfer plate should be kept on ice until the transfer of reagents to the Cartridge. **Note:** Ensure you prepare the Cell-free Blends last.

**3. eGene constructs (DNA)**

Take the vials or the plates with the eGene constructs made in advance using the eGene Prep Kit out of the freezer and thaw on the benchtop at room temperature. This takes approximately 15 minutes.

**Note:** the vials or the plates can be centrifuged for a few seconds to ensure all the liquid is at the bottom of the wells.

Load 5 µL of each eGene construct onto the transfer plate into wells: ▹ A1 to A8

▹ B1 to B8

▹ C1 to C8

**Note:** It is critical to load the eGene constructs onto the transfer plate in the exact order that they have been finalized in the experiment planned in the eProtein Discovery software.

**4. eProtein Discovery purification reagents** Thaw the Wash Buffer and the Elution Buffer on the benchtop at room temperature. Once thawed, vortex for 2 seconds and centrifuge for 2 seconds using a microcentrifuge to mix and collect all the reagents. ▹ Load 16 µL of Wash Buffer into wells C10 and D10.
▹ Load 16 µL of Elution Buffer into well F10
▹ Load 10 µL of ELution Buffer into wells H1 and H2

**5. eProtein Discovery controls** From the kit kept at -80°C, take the controls out and thaw them on ice.
▹ Load 10 µL of Blank Buffer into wells H3 and H4.
▹ Load 10 µL of Universal control into well H5.
▹ Load 10 µL of Complementation Control into well H6.
▹ Load 10 µL of Full W/F Control into well H7.
▹ Load 10 µL of Expression Control into well H8

**6. Strep Purification Beads**
Strep Purification Beads are provided in 200 µL aliquots of 5% v/v suspension – To prepare the Strep Beads:

1. Take the vial of Strep Beads from the fridge and give it a quick spin for 2 seconds in a microcentrifuge to pellet the beads.
2. Resuspend the beads by gently pipetting up and down 10 times with a p200 pipette set on 90 µL.
3. Transfer 90µL of the resuspended beads into a 1.5 mL tube. Discard the rest only after the experiment starts, in case more volume is required.
4. Place the tube with Strep Beads on a magnetic particle separator and capture for 1 min.

5. Remove all the supernatant with a p200 pipette and discard the liquid.
6. Remove the tube with Strep Beads from the magnetic particle separator. Resuspend the beads in 100 µL Wash Buffer by slowly pipetting up and down 10 times.
7. Repeat steps 4 to 6 twice more for a total of three washes.
8. After the third wash, spin down the tube and place it back on a magnetic particle separator and capture for 1 min.
9. Remove all the supernatant with a p200 pipette and discard the liquid.
10. Spin down the tube, place it back on a magnetic particle separator and remove the residual buffer with a p20 pipette.
11. With a p20 pipette, resuspend the beads in 10.5 µL Wash Buffer by gently pipetting up and down 10 times to create a 15 µL 30% Strep Beads working
12. Keep the beads in the tube on the bench, not on ice.
    **Note:** The beads should NOT be loaded onto the transfer plate.

**7. Detector Protein**

The Detector Protein is supplied as ready to use. Spin down the tube for 2 seconds to collect the full volume at the bottom. Load 16 µL of Detector Protein into wells A10, B10, G10, and H10 of the transfer plate.

**8. Preparation of the Cell-free Blends**

For each expression screening experiment, up to eight 20 µL Cell-free Blends can be made by adding 16 µL of Cell-free Core Reagent, 2 µL of a first additive, and 2 µL of a second additive.

**Note:** The total volume of blend should always be 20 µL final
**Note:** the same additive can be used as first and second additive, for example 2 x 2 µL of Additive Buffer. The list of Additives is in Table 1.

1. Thaw on ice Cell-free Core Reagents and Additives

2. Once thawed, vortex for 2 seconds the Cell-free Core reagents and Additives to ensure they are well mixed.
3. Centrifuge for 2 seconds the Cell-free Core reagents and Additives using a microcentrifuge to return any droplets to the bulk aliquot.
4. Add 16 µL of Cell-free Core reagent to wells A12-H12.
5. Add 2 µL of your first selected additive to wells A12-H12.
6. Add 2 µL of your second selected additive to wells A12-H12.

**Note:** It is critical to load the Cell-free Blends onto the transfer plate in the exact order that they have been finalized in the experiment planned in the eProtein Discovery software.

## Set up the experiment on the instrument

Press the **[Power Switch]** to activate the Instrument power-up, initialization and self-test sequence.

### Create the experiment

1. From the top right hand side of the screen select **[Create Experiment]**
2. Select the **'eProtein Discovery Screen Experiment'** workflow and press **[Confirm]** (Figure 8)

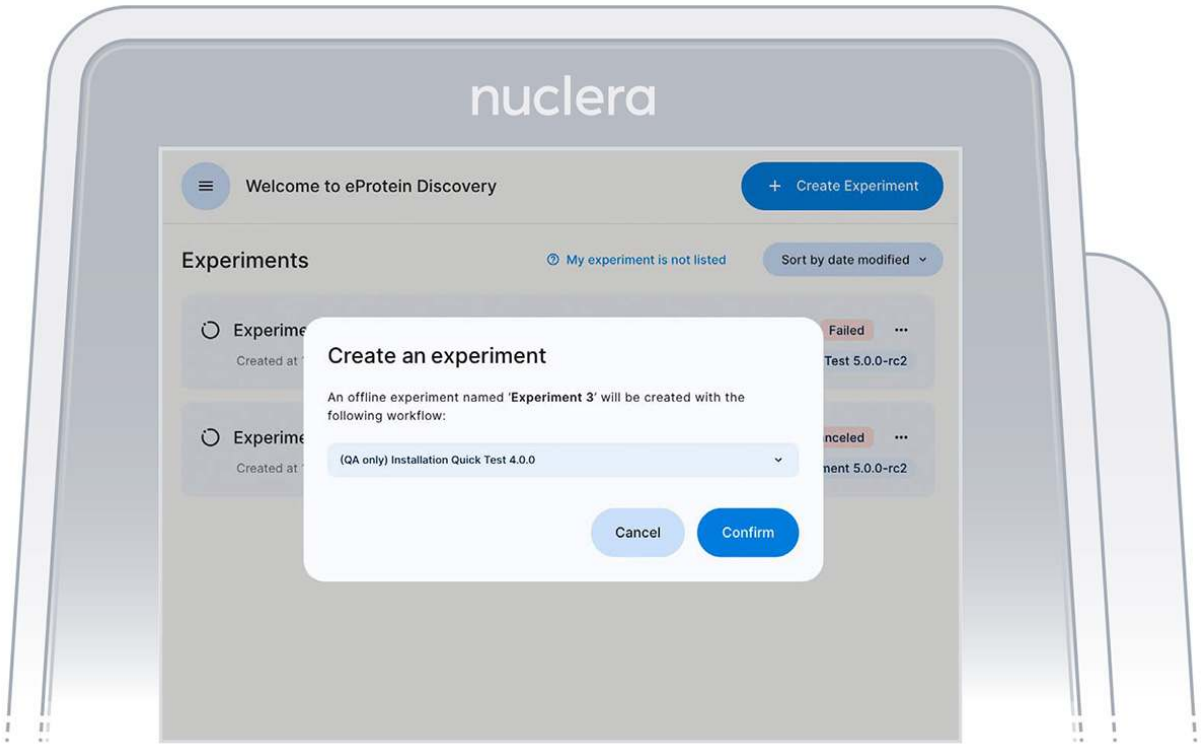



**Figure 8: Experimental design screen**

3. Select the type of experiment you would like to perform on the instrument (Figure 8). You can choose between:

    a. 3x8 (3 proteins, 8 solubility tags)
    b. 4x6 (4 proteins, 6 solubility tags)
    c. 6x4 (6 proteins, 4 solubility tags)
    d. 24x1 (24 proteins, FlexiVariant screen)
    e. 30 highest expressing combinations

**IMPORTANT NOTE**

It is critical to select the exact experiment format as this will determine the downselection method of the 30 expression conditions for the strep purifiability assessment.

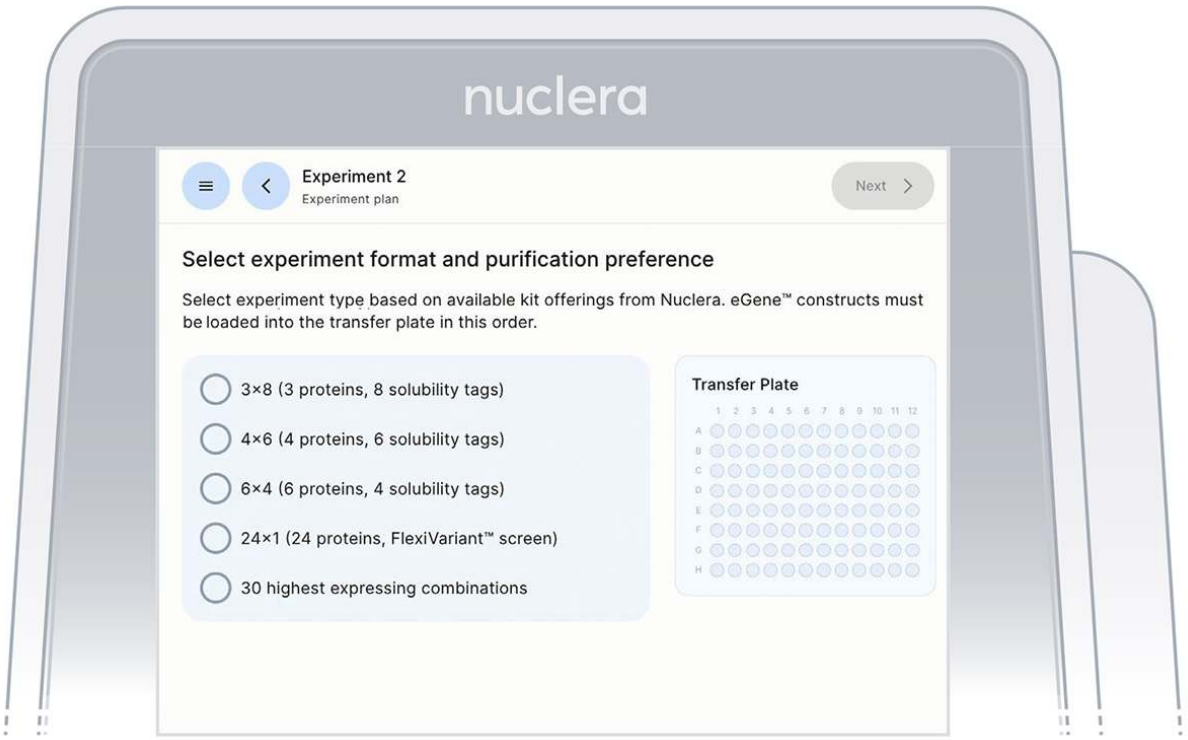



**Figure 9: Instrument screen to select the experiment format**

4. Read the Before you proceed section and press the [Next] button (Figure 10).

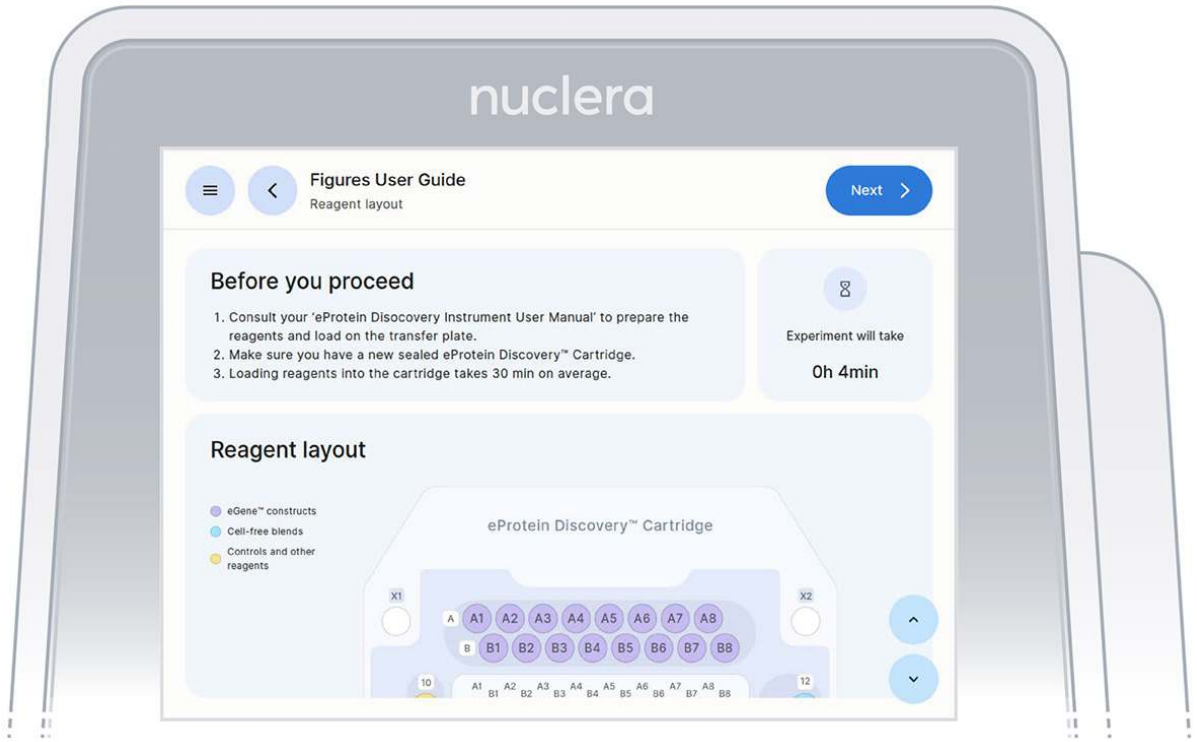



**Figure 10: Summary of the selected experiment**

5. Get the transfer plate containing the reagents and cartridge ready.
6. Go through and tick the checklist, and press the **[Next]** button (Figure 11). The drawer will open.

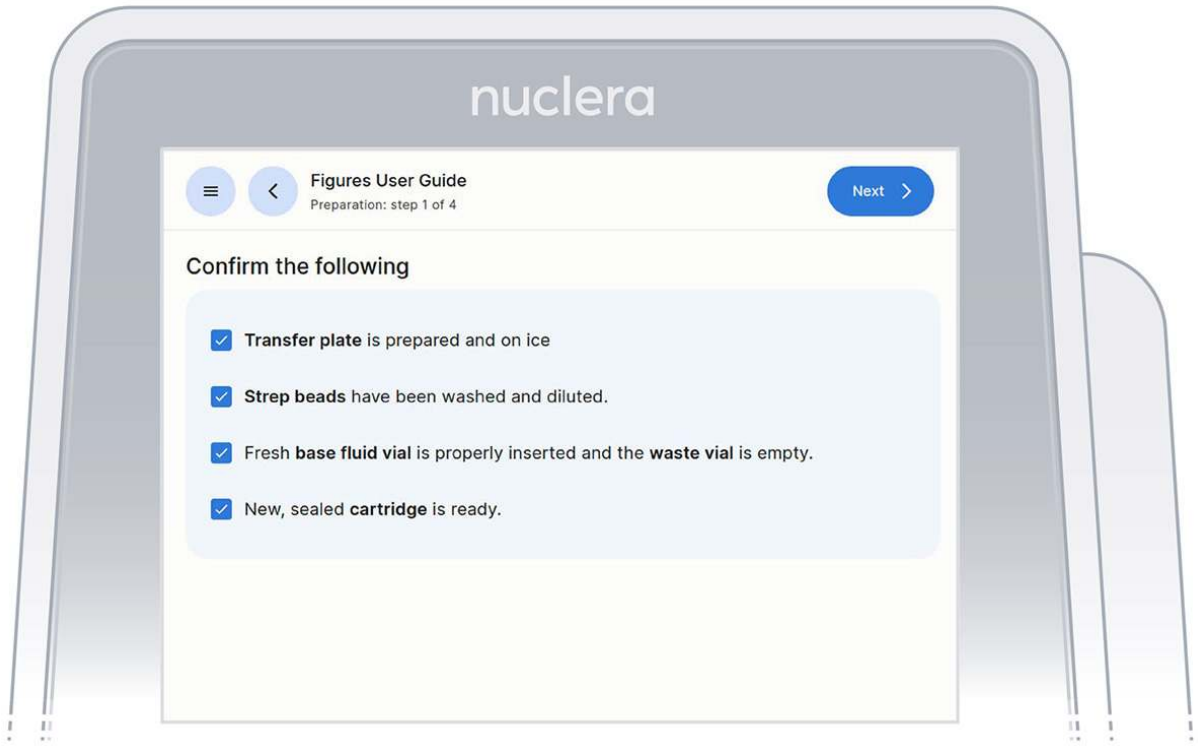



**Figure 11: Checklist screen before the experiment starts**

7. Unpack and load a cartridge as shown on the screen of the eProtein Discovery instrument, place the cover on the cartridge, avoid touching the electrical connectors, and press the **[Next]** button (Figure 12).

**Note:** keep the cartridge packaging to dispose of the cartridge after use.


**Figure 12: Loading of the cartridge on the eProtein Discovery instrument**

8. Keep cover on the cartridge. Markings on the cover will guide you through the loading process.

## Set up the pump on the instrument

**Follow the on-screen instructions to complete the experiment.**

▹ These instructions will guide you in operating the eProtein Discovery instrument and completing an experiment on the instrument.

▹ The instructions must be followed in the order shown on the screen.

▹ You can navigate forward and back through the steps using the buttons at the top.

▹ You can scroll up and down using the arrows at the bottom right of the screen when shown or with your fingers.

**Note:** once you start the experiment, the back button on the instrument

will be disabled.

1. On the right hand side of the instrument, ensure the tubings for the integrated pump are placed in the tubing holder, and press the **[Next]** button (Figure 13).


**Figure 13: Loading of the cartridge on the eProtein Discovery instrument**

2. Ensure the vial of base fluid and the waste container have been connected to the pump located on the right hand side of the instrument. Press the **[Next]** button (Figures 14).

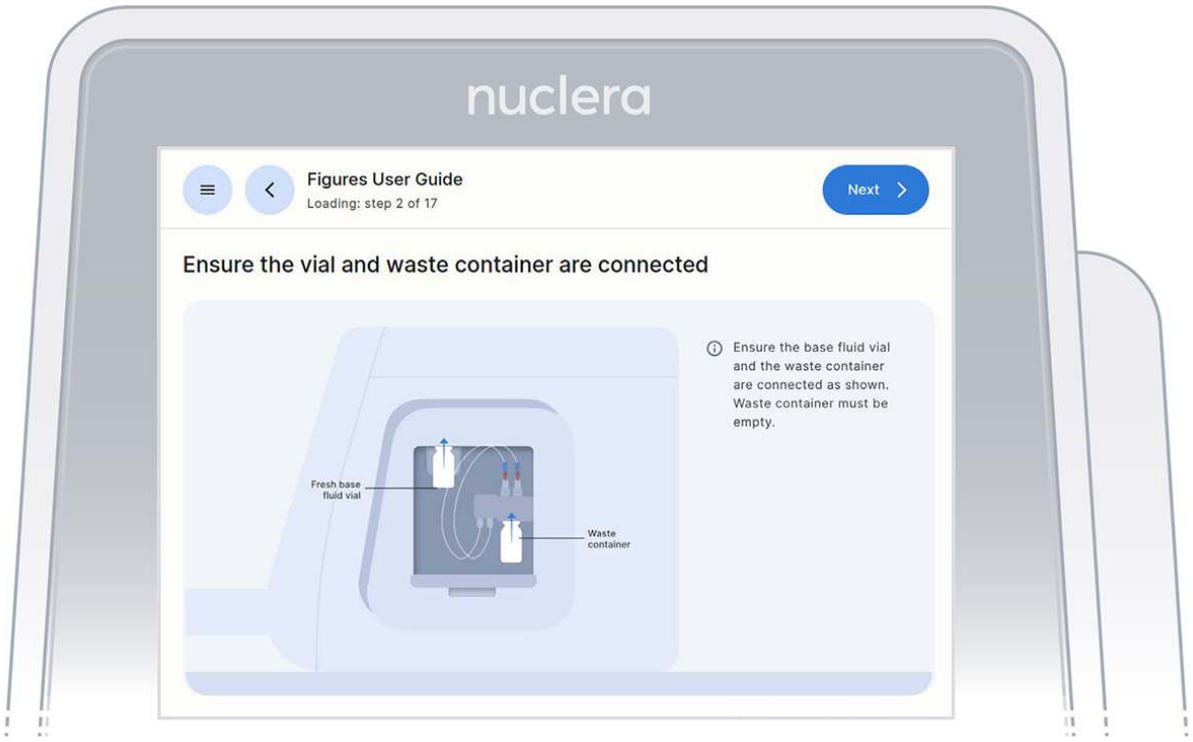



**Figure 14: Vial of base fluid and the waste container connected to the pump as shown on the screen**

## Filling the cartridge with base fluid

1. With the tubing and containers in place, ensure that some of the base fluid has dripped into the waste container (Figure 15).

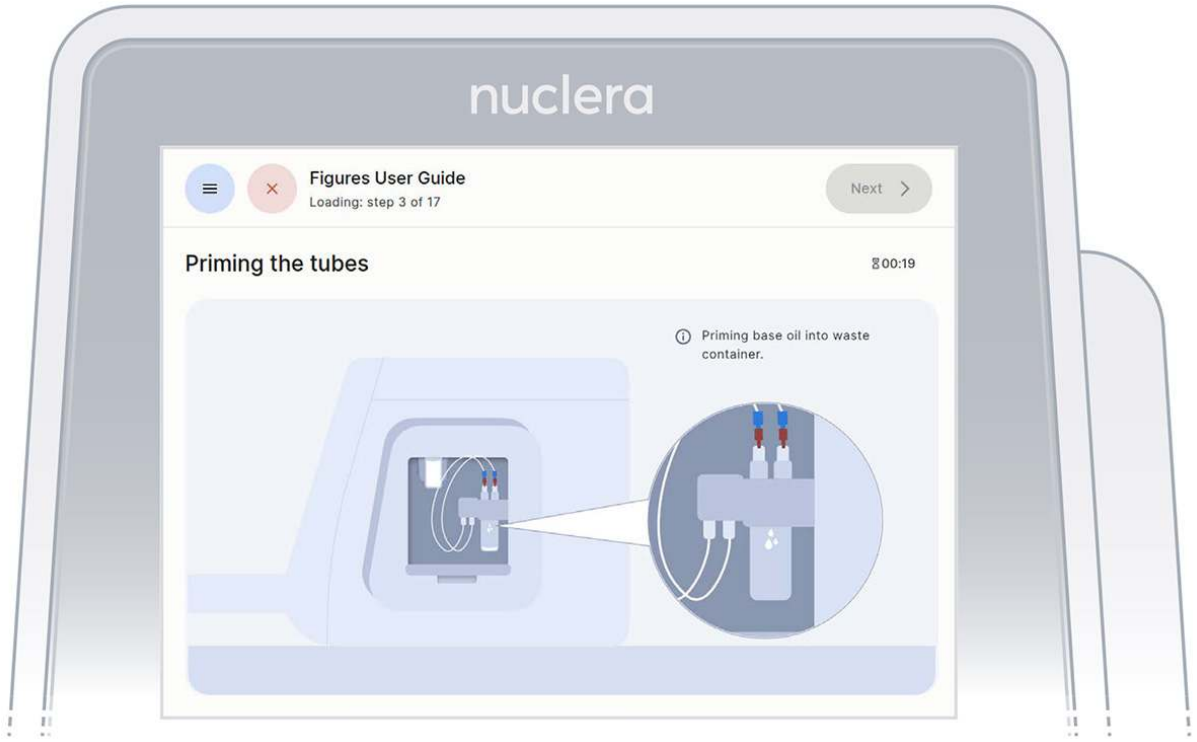



**Figure 15: Priming the pump tubings with base fluid**

2. Remove connectors tubing from the holder, connect them tightly to the corner ports X2 and X3 of the cartridge, and press the **[Next]** button (Figure 16). Either connector can be interchangeably inserted into corner port X2 or X3.

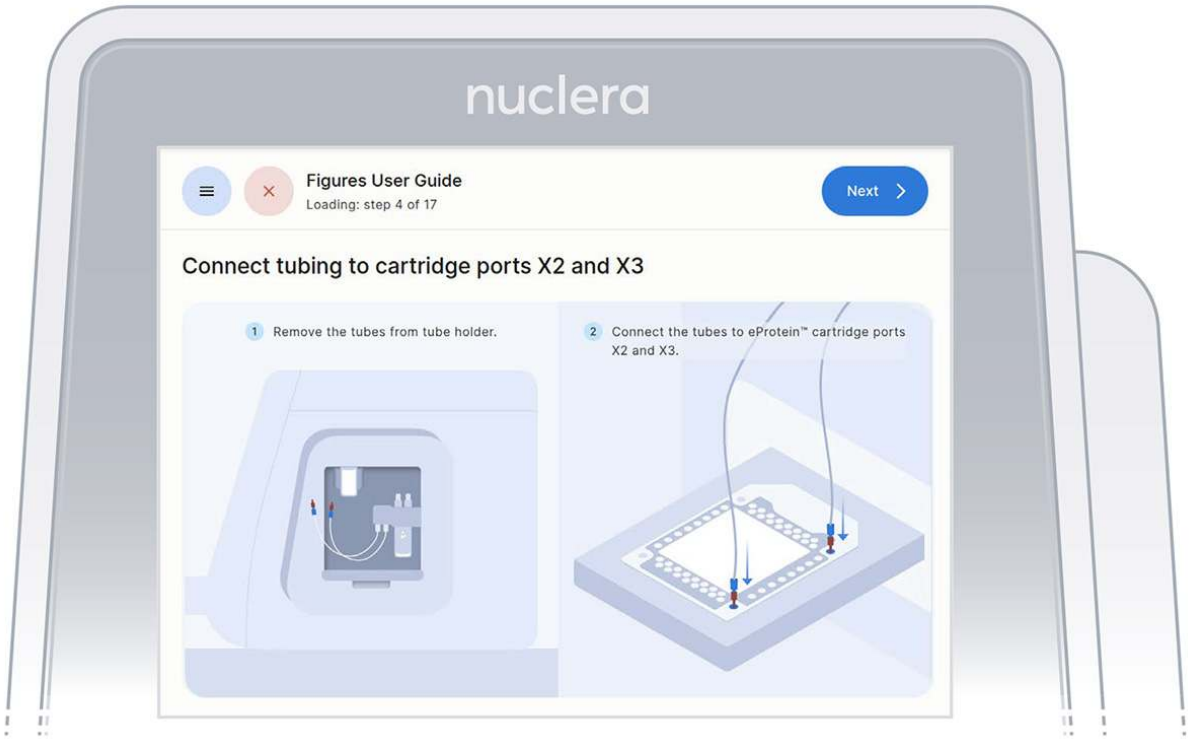



**Figure 16: Connection of the pump tubes to the cartridge**

3. Inspect the cartridge for air bubbles that may have been introduced during the priming with base fluid.
   If any air bubbles persist after base fluid priming, use a single-channel p200 pipette to aspirate the air bubbles from the nearest port and reinject slowly the base fluid that was aspirated into a corner port (X1 or X4). Press the **[Next]** button (Figure 17).

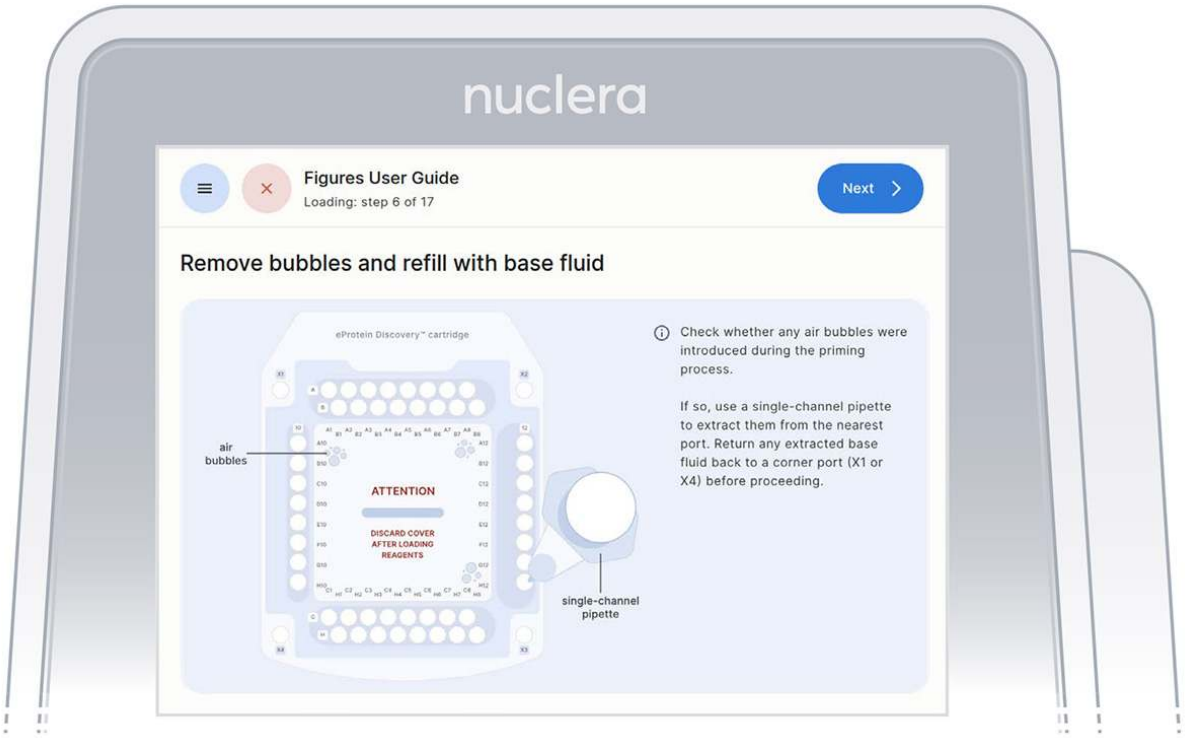



**Figure 17: Procedure to remove potential air bubbles from the cartridge**

4. Inspect the ports on the cartridge after the priming with base fluid is complete. Ensure all the ports are filled and press the **[Next]** button (Figure 18).

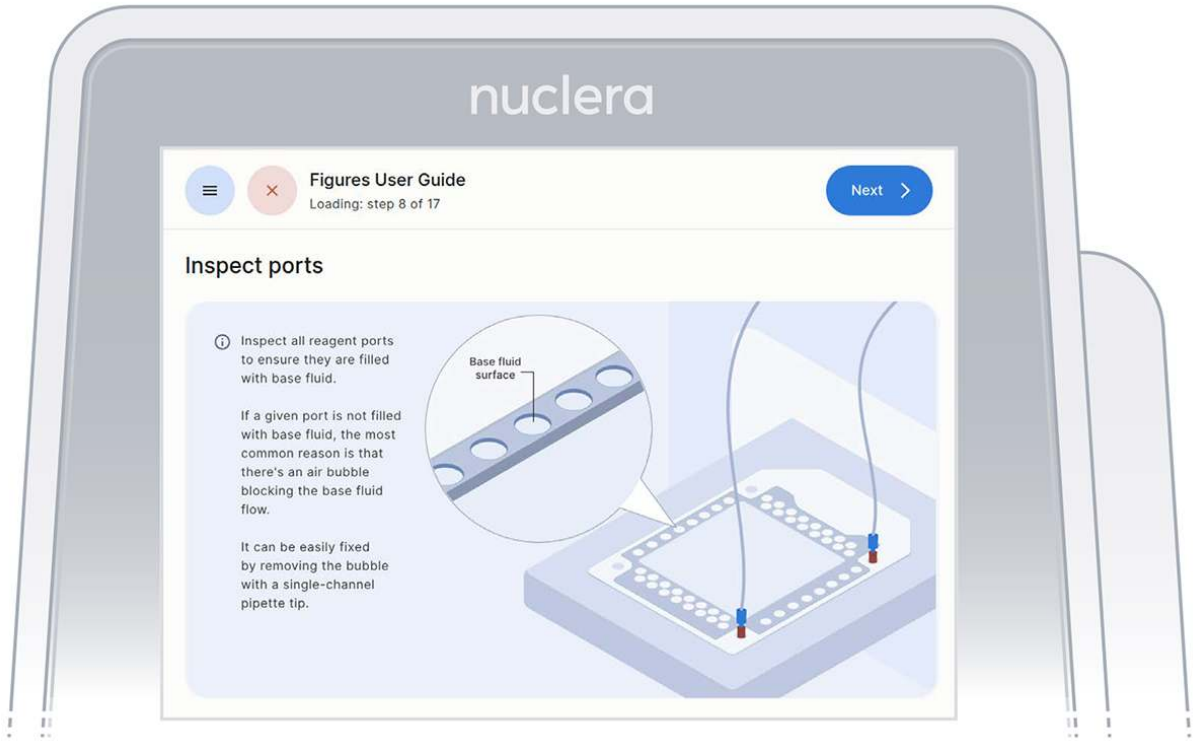



**Figure 18: Procedure to remove potential air bubbles from the cartridge**

## Load the reagents on the cartridge

**Note:** videos showing how to load the reagents on the cartridge can be found on the Nuclera website via this link: www.nuclera.com/resource-library/?resource_type=video.

### Guidance for proper sample loading:

▹ Follow the on-screen instructions that will guide you in loading the reagents.
▹ The loading of the reagents should be done using a volume appropriate 8-channel pipette.
▹ To facilitate the pipetting of the reagents, the transfer plate can be moved from the ice bucket to the bench.
▹ Check the plate for the presence of air bubbles. Air bubbles can be

removed by spinning the plate in a swing rotor centrifuge for about 10 seconds.

▹ After aspirating the reagents, make sure that all pipette tips are filled evenly, and contain no air bubbles.

▹ When loading the reagents into the ports, place the tip vertically in the port, just under the meniscus of base fluid. Do not touch the sides or base of the port.

▹ Dispense reagent gently to the first pipette stop. Do not go past the first stop.

▹ After dispensing, lift the tip while keeping the pipette plunger depressed. If using an electronic pipette, make sure that the purge function is disabled.

▹ Lift the tip while keeping the pipette plunger depressed.(Figure 19).

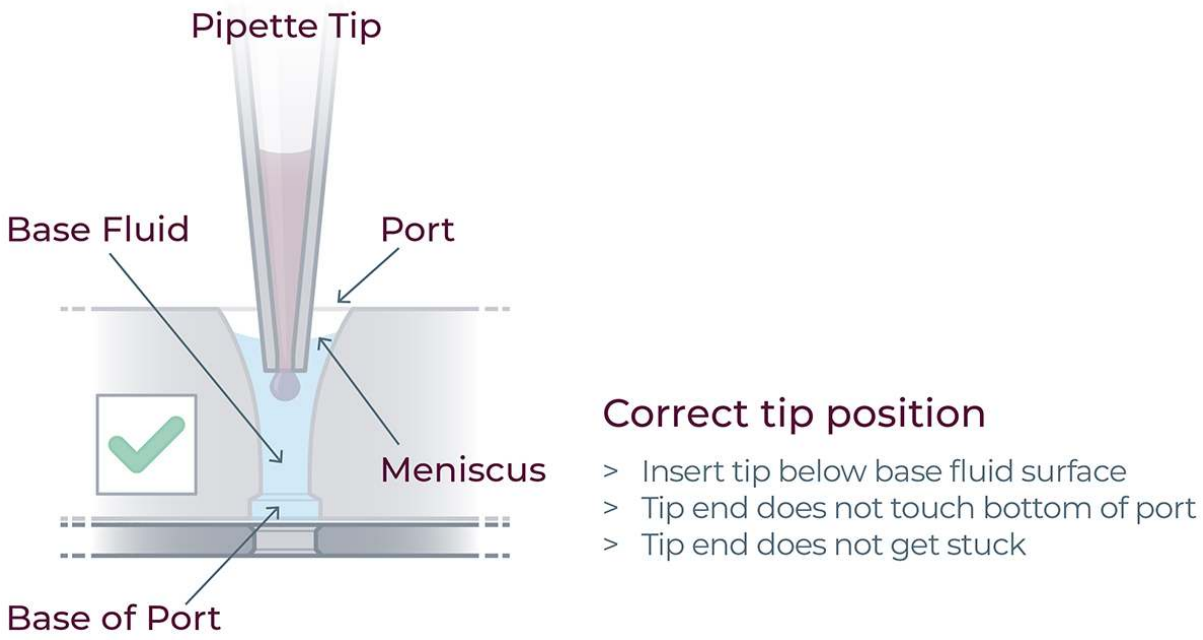



**Figure 19: For correct reagent loading the pipette tip is immersed in the base fluid and not touching the bottom of the port**

**IMPORTANT NOTE**

Electronic pipettes must be properly configured for use with eProtein

Discovery. If you are using an electronic pipette, please contact Technical Support at techsupport@nuclera.com for assistance. Any run errors in eProtein Discovery caused by incorrect use of electronic pipettes are the responsibility of the user. We recommend using a manual multichannel pipette instead. Important settings to note for electronic pipettes are:
▹ Disable blowout/purge
▹ Avoid high speed for aspiration
▹ Avoid high speed for dispense.

## Load eGene constructs - rows A, B and C

**IMPORTANT NOTE**

It is critical not to leave any port empty. If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit, **not with water.**

**Tip:** Empty ports can be used for duplicates.

▹ Load 8x fresh pipette tips and aspirate 3 µL of the eGene constructs from the transfer plate wells A1-A8 into ports A1-A8 of the cartridge (Figure 20).
▹ Load 8x fresh pipette tips and aspirate 3 µL of the eGene constructs from the transfer plate wells B1-B8 into ports B1-B8 of the cartridge (Figure 20).
▹ Load 8x fresh pipette tips and aspirate 3 µL of the eGene constructs from the transfer plate wells C1-C8
▹ into ports C1-C8 of the cartridge (Figure 20). ▹ Place the tip vertically in the port, just under the meniscus of base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.

▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.

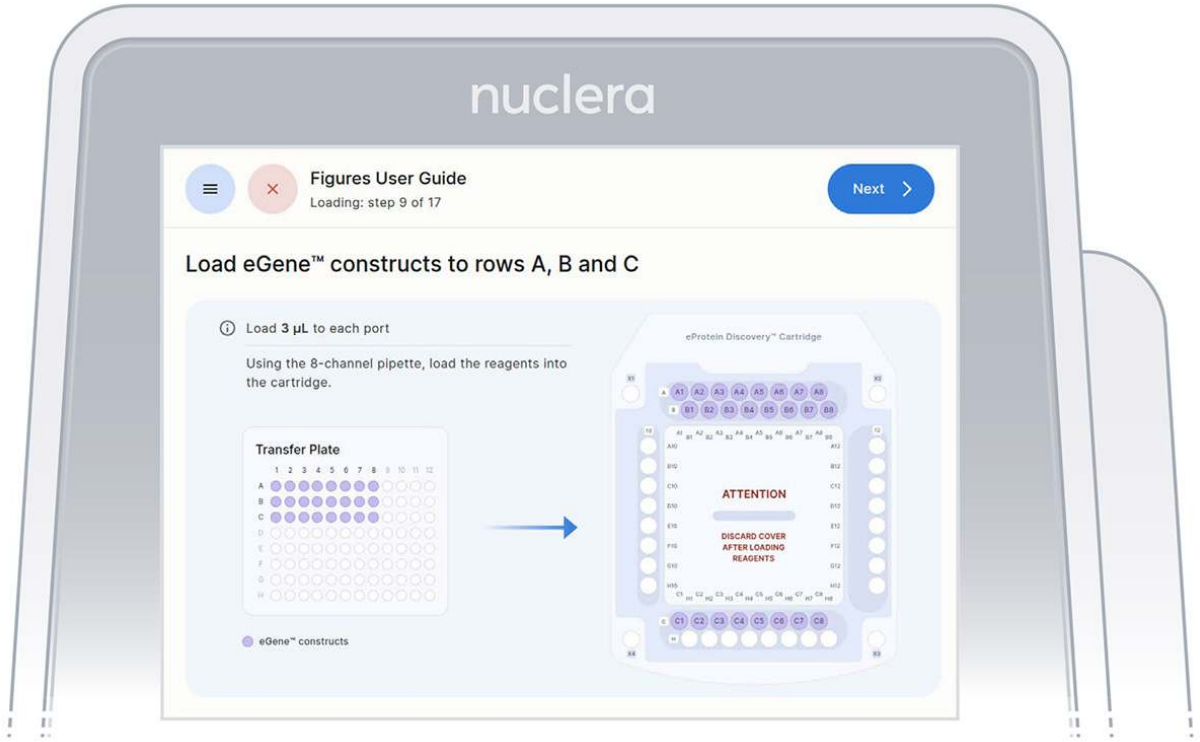



**Figure 20: Loading of the eGene constructs onto row A, B and C of the cartridge**

# Load reagents - row H, column 12 and column 10

**1. Reagents - row H:**

▹ Load 8x fresh p20 pipette tips and aspirate 3 µL of the reagents from the transfer plate wells H1-H8 into ports H1-H8 of the cartridge (Figure 21).
▹ Place the tip vertically in the port, just under the meniscus of base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.

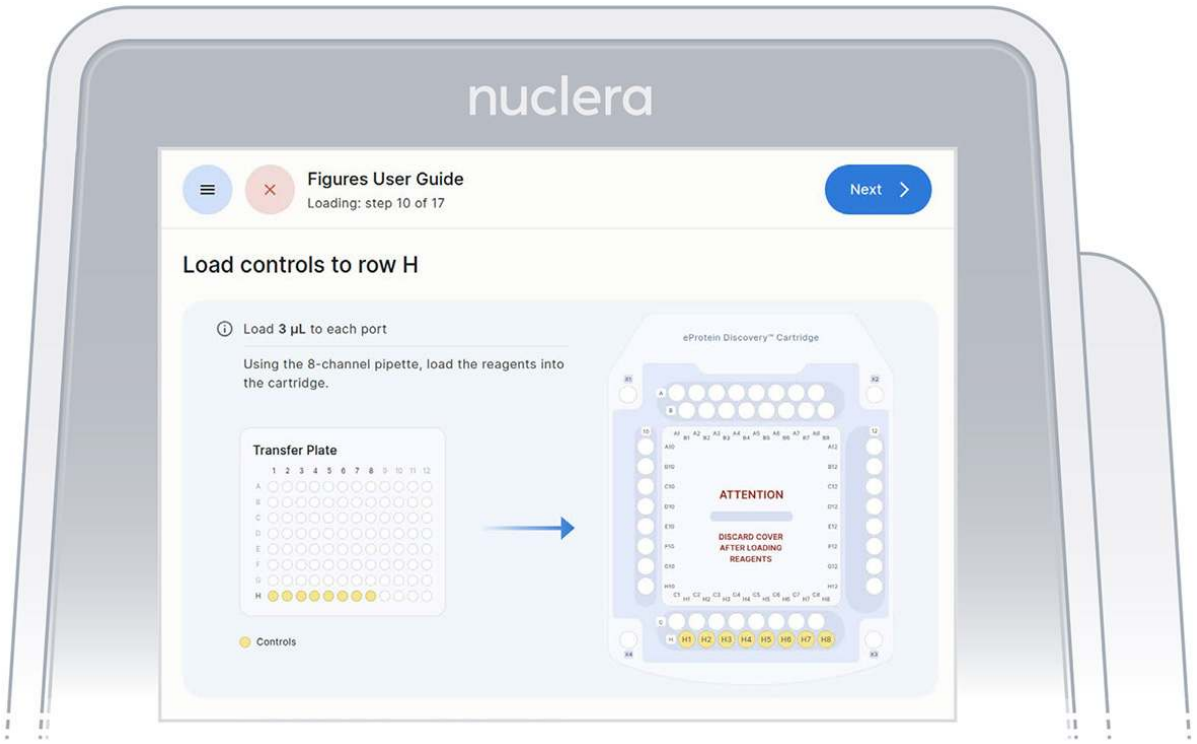



**Figure 21: Loading of the reagents onto row H of the cartridge**

### 2. Reagents - column 12:

▹ Load 8x fresh p20 pipette tips and **mix the Cell-free Blends in the transfer plate by gently pipetting up and down 12 times. **

**IMPORTANT NOTE**

Be careful not to introduce air bubbles in the ports.

**IMPORTANT NOTE**

Aspirate 12 µL of the Cell-free Blends from the transfer plate wells A12-H12 into ports A12-H12 of the cartridge (Figure 22).

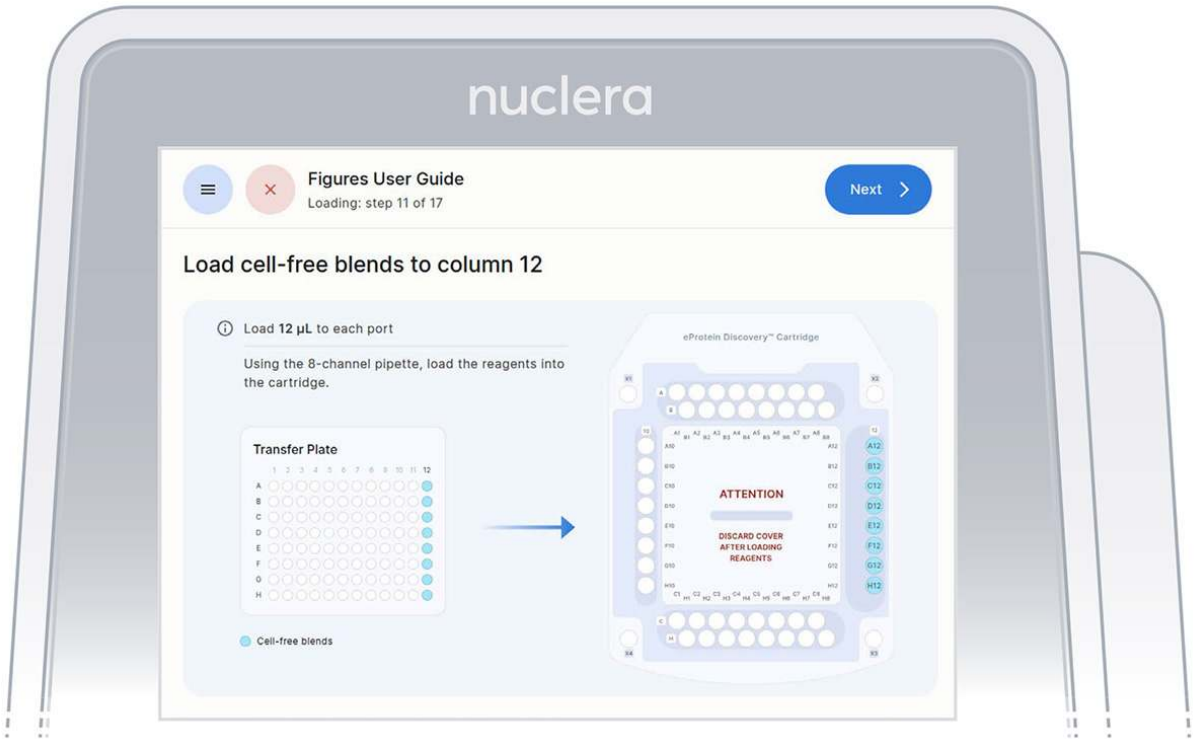



**Figure 22: Loading of the Cell-free Blends onto column 12 of the cartridge**

▹ Place the tip vertically in the port, just under the meniscus of base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.

**3. Reagents - column 10:**

▹ Load 7x fresh p20 pipette tips and aspirate 12 µL of the reagents from the transfer plate wells A10-D10 and F10-H10 into ports A10-D10 and F10-H10 of the cartridge (Figure 23).
▹ Ensure the tip is immersed in the base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tips fully into the ports.
▹ Eject the pipette tips into a waste container.
▹ Press the **[Next]** button on the screen.

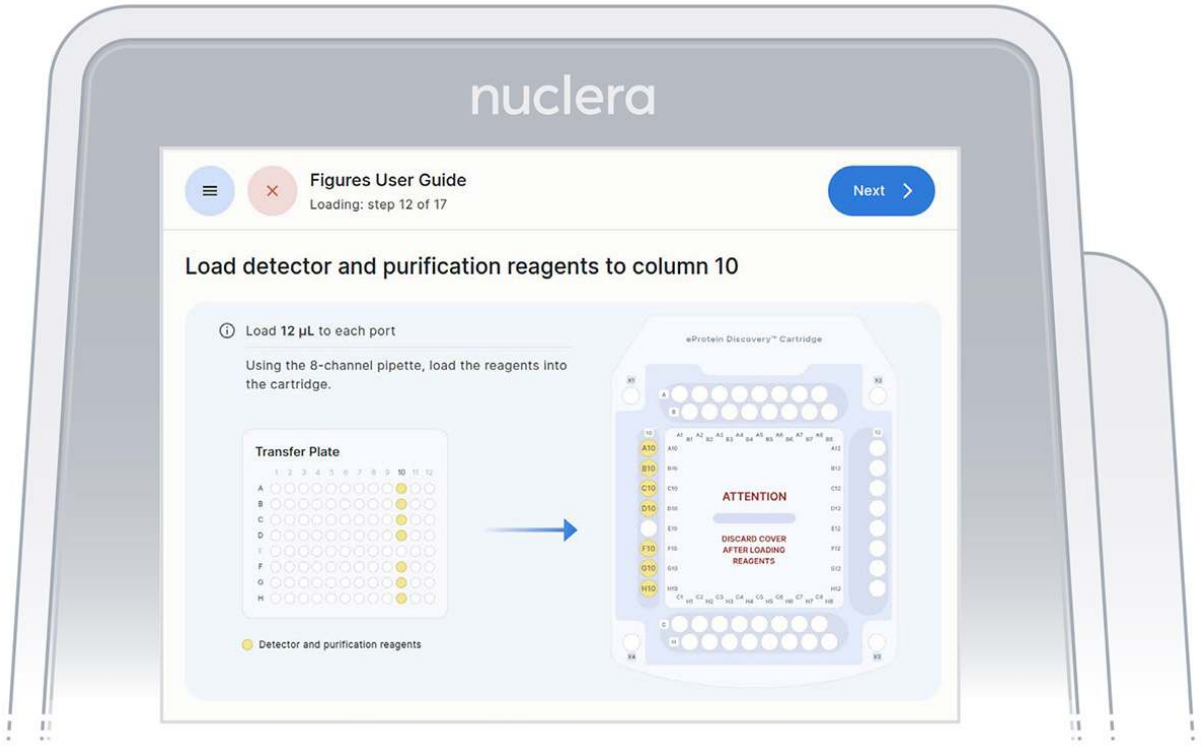



**Figure 23: Loading of the reagents onto column 10 of the cartridge**

### 4. Strep Purification Beads - port E10:

▹ Using a single channel p20 pipette, mix the Strep Purification Beads twelve times by gently pipetting up and down. Be careful not to introduce air bubbles. Aspirate 12 µL of the Strep Purification Beads prepared in a tube and dispense into port E10 of the cartridge (Figure 24).

▹ Place the tip vertically in the port, just under the meniscus of base fluid and dispense slowly until the first stop of the pipette is reached. Do not engage the pipette tip fully into the port.

▹ Eject the pipette tip into a waste container.

▹ Press the **[Next]** button on the screen.

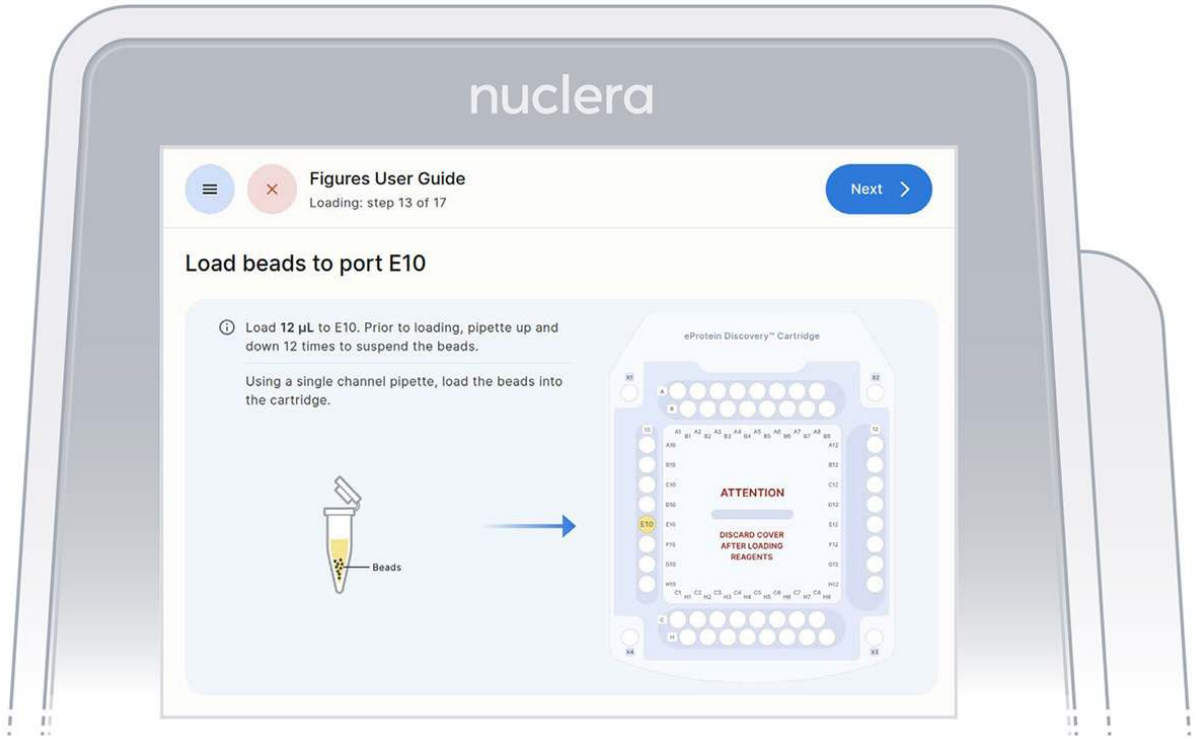



**Figure 24: Loading of the Strep beads to port E10 of the cartridge**

5. Remove the cover from the cartridge (Figure 25)

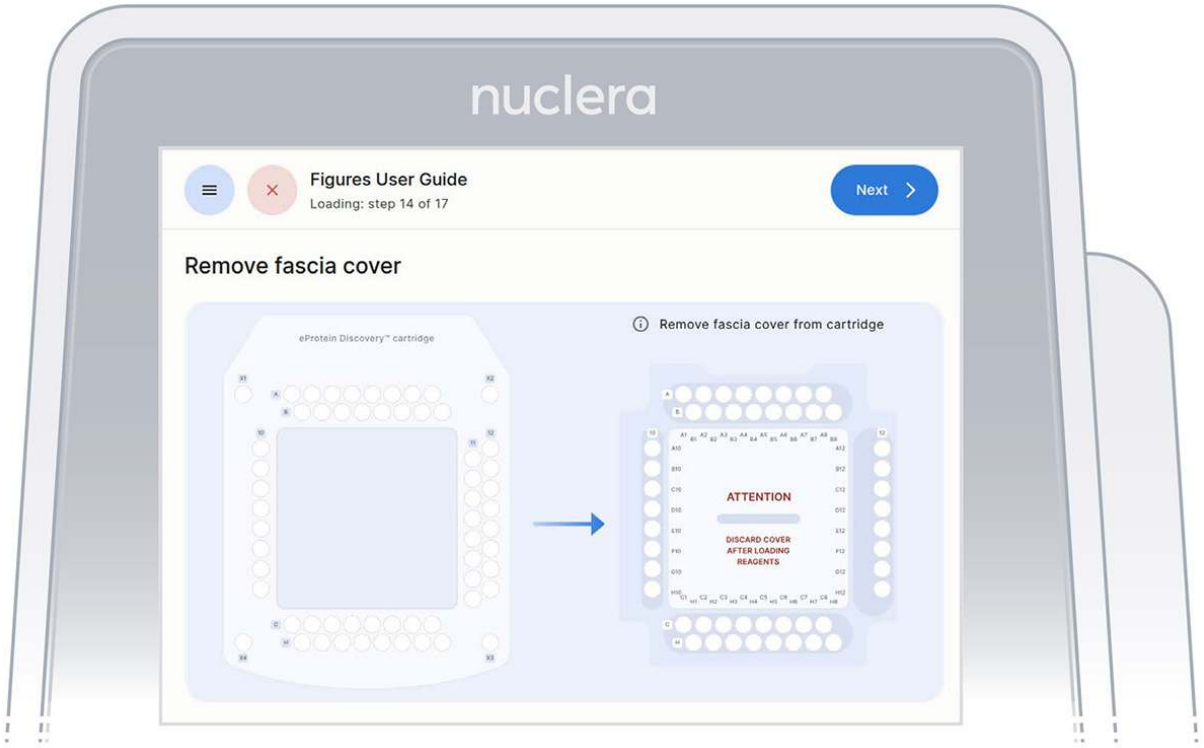



**Figure 25: Remove the cover from the cartridge**

## Load reagents in the cartridge

1. Press the **[Next]** button to start the aspiration of the base fluid and the loading of the reagents on the cartridge (Figure 26).

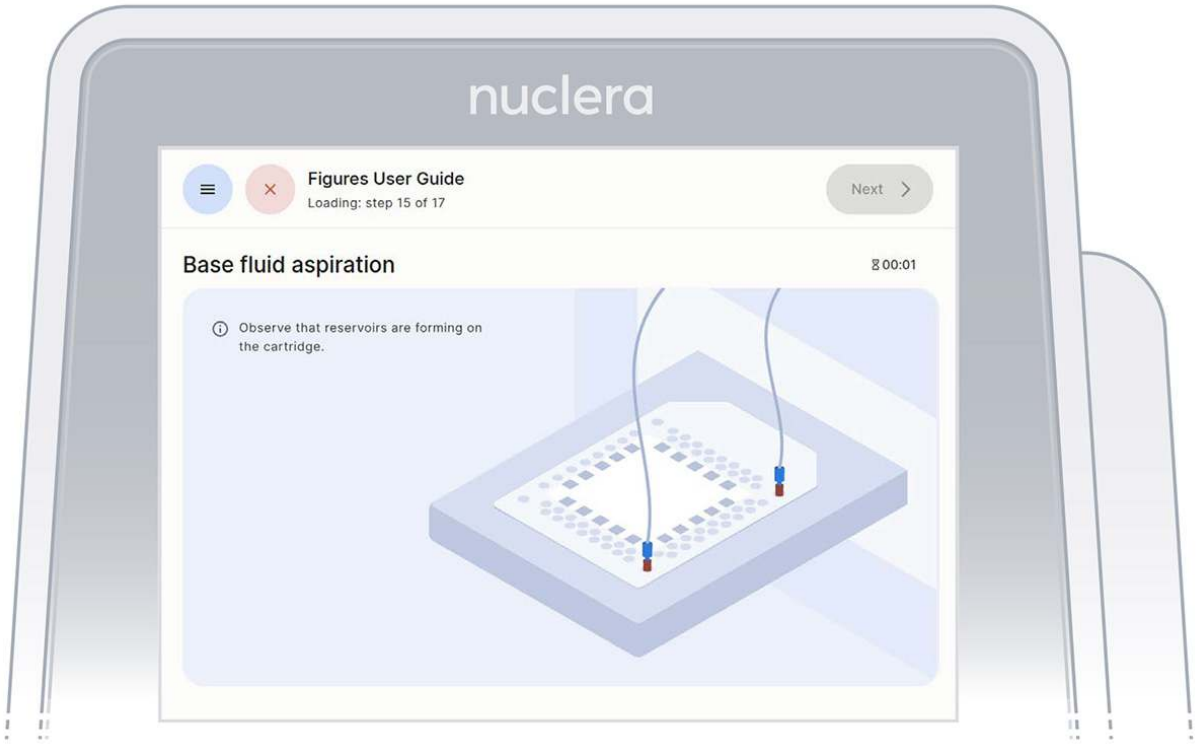



**Figure 26: Base fluid aspiration**

2. Disconnect the tubes from the cartridge and place them in the tube holder on the right hand side of the instrument. Press the **[Next]** button on the screen (Figure 27), and the drawer will close. Loading checks will be performed, and the drawer will open.

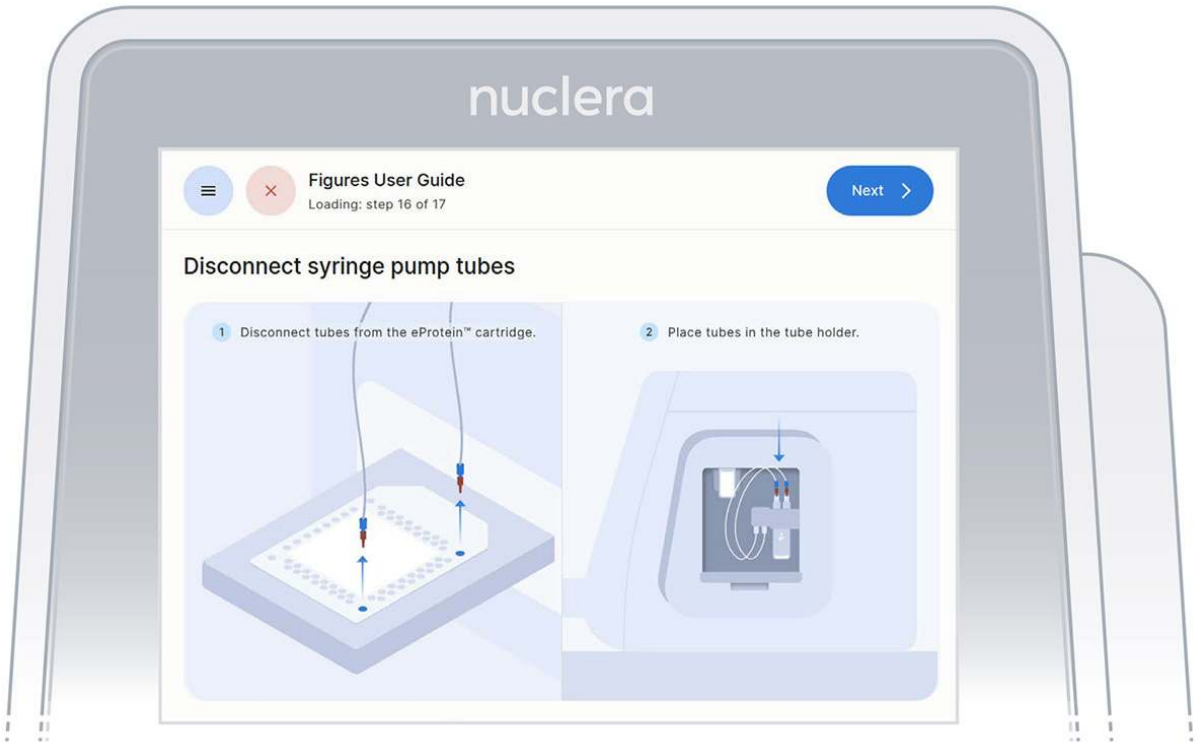



**Figure 27: Disconnect the tubes and place them on the tube holder**

3. Inspect the reservoirs have formed correctly in the cartridge as shown on the screen. If so, Press the **[Next]** button (Figure 28).
   **Note:** Any presence of a marker on the reservoir is acceptable, as shown on the top right of Figure 29

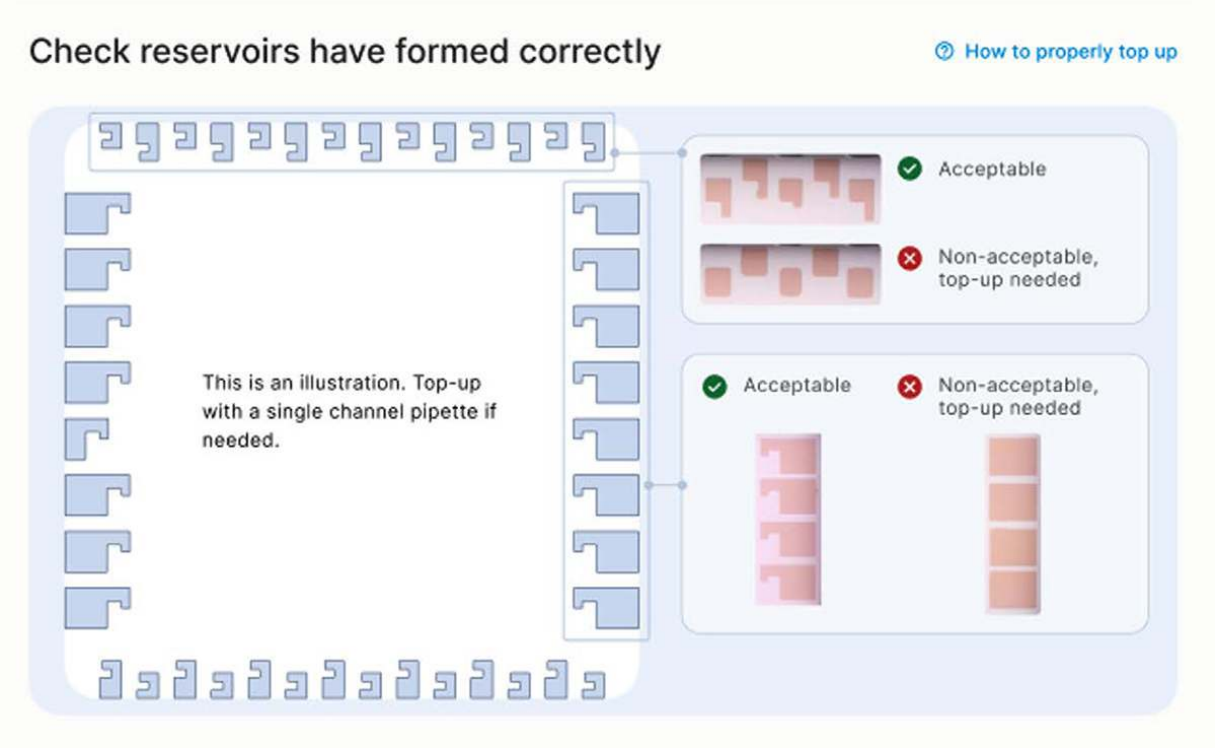



**Figure 28: Check the reservoirs have formed correctly on the cartridge**

### Troubleshooting tip 1:

If a reservoir is not properly formed, first fully engage a new empty pipette tip into the port and reach the bottom of the port. This action may trigger the correct formation of the reservoir.

### Troubleshooting tip 2:

▹ If the shape of a reservoir is still not correct, remove the empty tip from the port, replace with a new tip, then add a small volume of the corresponding reagent using a p20 pipette with a pipette tip. Do not depress the pipette past the first stop as this could introduce air bubbles inside the cartridge.

▹ Re-engage the tip until reaching the bottom to the port and dispense the reagent slowly until correction is complete (Figure 29).

▹ The recommended volumes for manual correction are:

▹ 1.5 µL for ports in rows A, B, C or H

▹ 3 µL for ports in columns 10 or 12

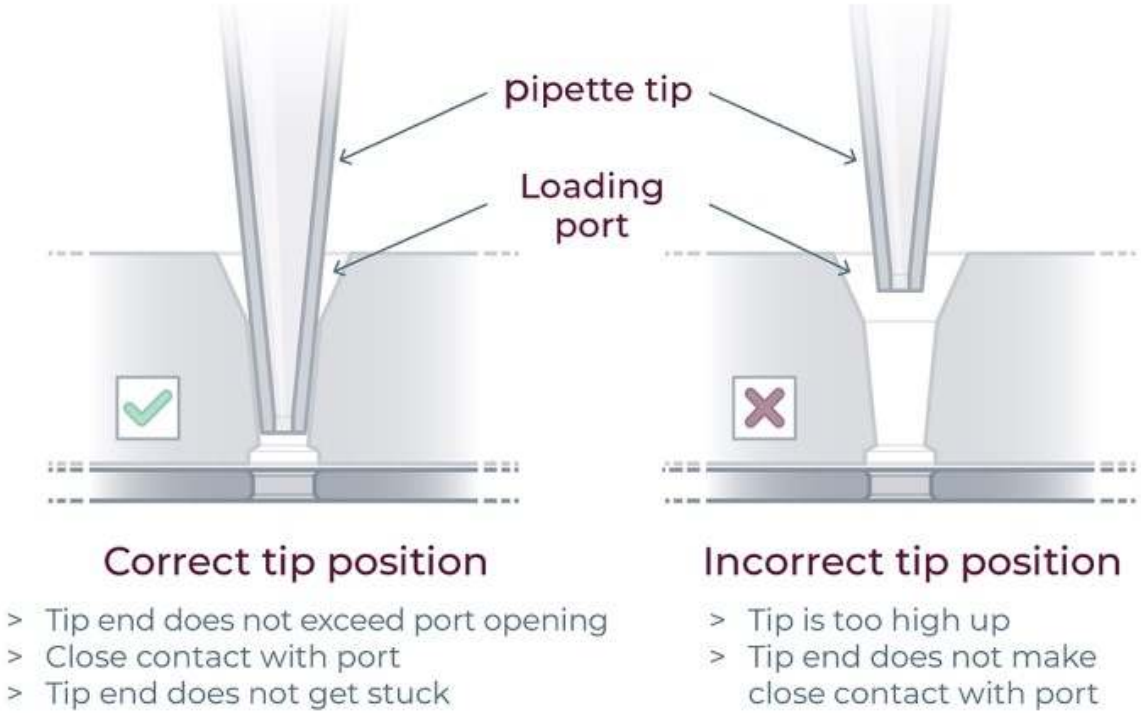



**Figure 29: For manual correction of the reagent loading to correctly form the reservoirs on cartridge**

Once the experiment starts, it will take approximately 24 hours to complete. You can monitor its progress on the screen.

# Analyze the results

### Instrument software results screen

After completion of the experiment, the results are shown on the instrument screen. The four best obtainable combinations of eGene and Cell-free Blend are displayed with the predicted in-tube scale-up yields (Figure 30).

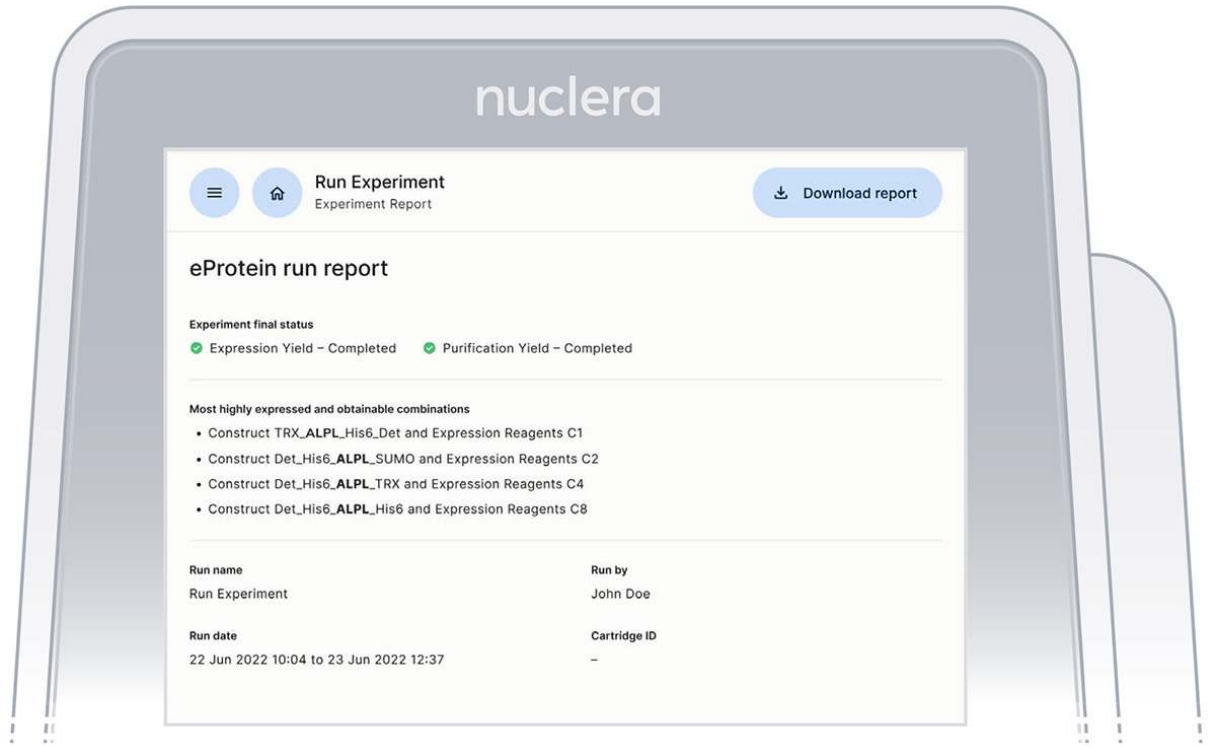


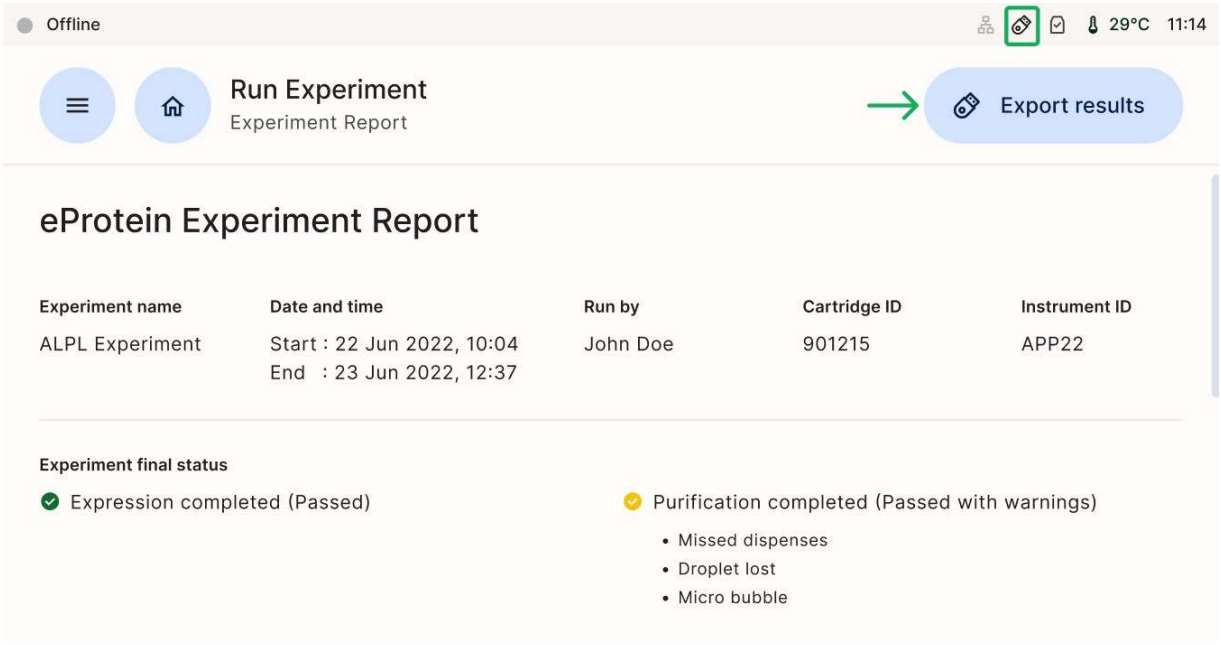

**Figure 30: Result screen from the Instrument Software**

# eProtein Discovery report

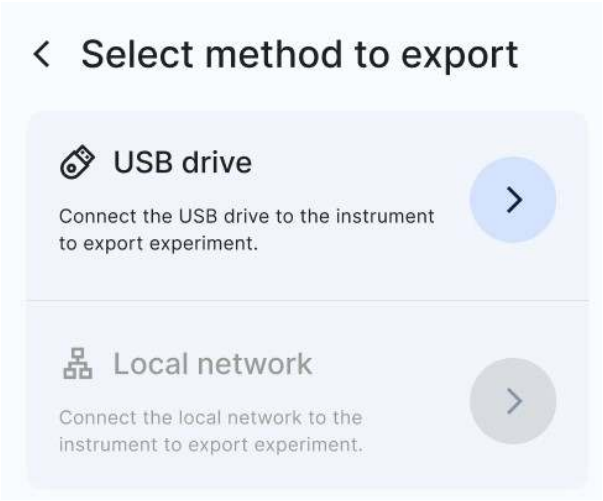
At the end of the experiment a report containing all the information about the experimental setup and the results can be downloaded from the instrument. Data can be exported using a USB, LAN or a Local Laptop. The upload takes about 15 minutes and during this time the [Download Report] button at the top right corner of the screen is grayed out.

### USB

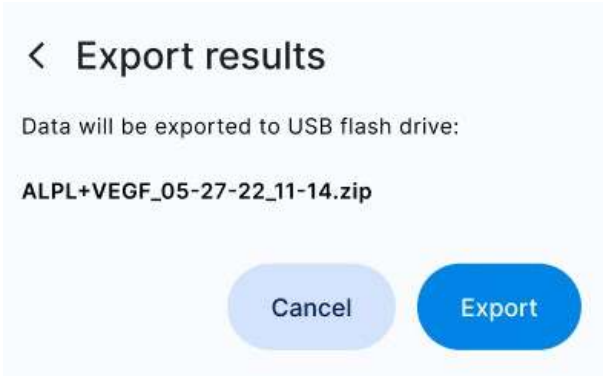
To export data to a USB flash drive it required to have a company-approved USB flash drive for data transfer. Encrypted flash drives are not currently supported. Insert the flash drive on the right hand side of the instrument in the USB port. This will highlight the flashdrive icon on the screen. Click on **[Export Results]**




A selection Window will show the two options available. Select (a) **USB drive** and click on (b) **Export** in the next window as shown in figure below.




(a)

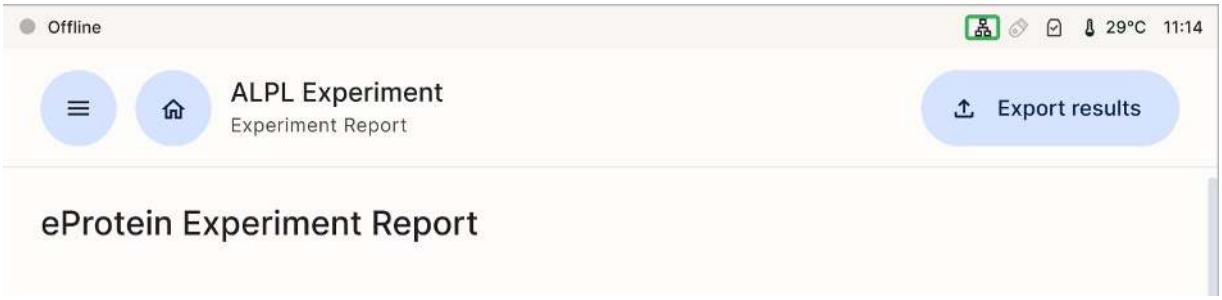



(b)

Data is retrieved from the instrument by inserting a USB flash drive into the instrument and exporting the data onto it, then inserting it into a Windows laptop and using an Excel spreadsheet to analyze the data. For data visualisation and analysis, it is required to have a laptop with Microsoft Excel

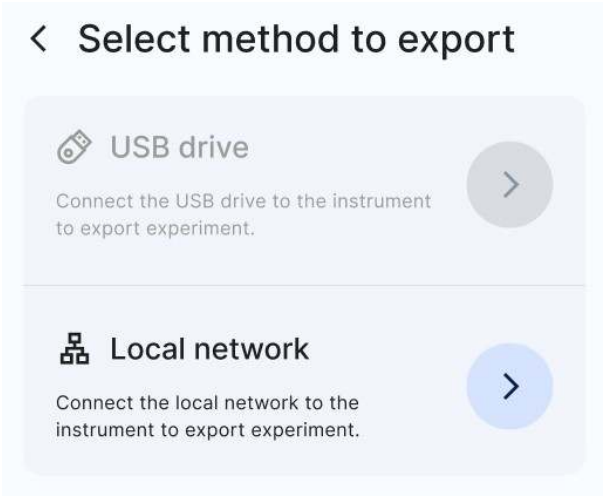
### LAN and Local Laptop

Connect an Ethernet cable directly from the instrument's Ethernet port to the Windows laptop (or to the Ethernet-to-USB dongle). On the instrument: Check that the LAN icon is no longer greyed-out.

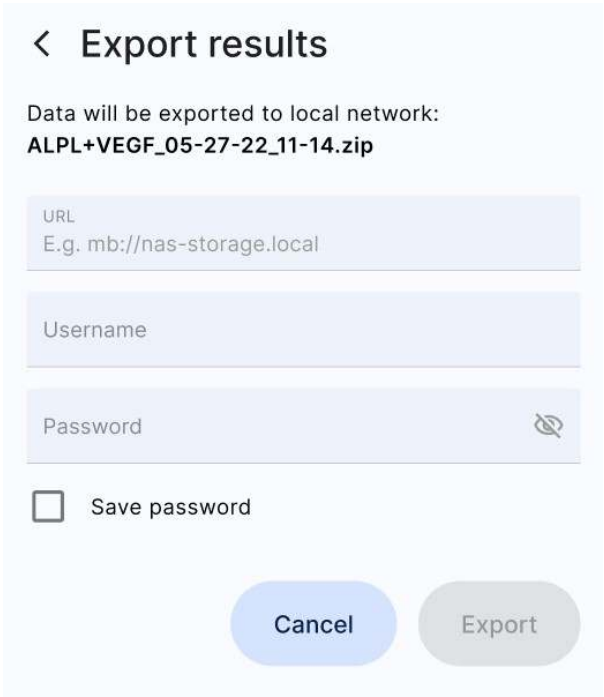



Open a completed experiment and tap the “Export results” button.
Tap (a) **"Local network"** and enter the following (b):
**URL**: smb://192.168.1.100/eProteinReports
**Username**: the name of the Windows user that has access to the shared directory, if this is a domain user then the username should be of the form DOMAINNAME\username
**Password**: the password of the Windows user that has access to the shared directory

(a)




(b)




**Note:** the instrument should not be switched off until the report is transferred.

**The experiment report contains:**

▹ **Experiment video**

The experiment video provides a record of cartridge droplet operation, allowing users to review performance for quality control, troubleshoot issues, or verify specific events during the run. Any questions or concerns regarding the operation of the droplets should be directed to the Nuclera

Technical Support team (techsupport@nuclera.com).

▹ **PDF report file**
The PDF report file is a summary of the experiment setup and the results, saved in the report folder with the name given to the experiment included in the file name

▹ **CSV report file** The report file is a csv file saved in the report folder with the name given to the experiment included in the file name. The results for each one of the 30 purified target protein conditions, and the 192 conditions for the produced protein are listed in the csv file. It also contains the measured values for the controls, the expected range for the controls, and a PASS/FAIL score if the measured values are within the expected range.

▹ **Blue light images (TIFF images)**
Images acquired at the end of expressions and purification. These images can give the user information about the solubility of the protein.

▹ **Other files**
The folder contains additional files that can be used by the Nuclera Technical Support team for troubleshooting purpose.

## Visualise your data

1. Unzip (right click + Extract All) your report folder exported from eProtein Discovery instrument
2. Open the report.csv file and select the entire sheet by clicking the top left corner, or by using the shortcut "Ctrl + A" or "Cmd + A".
3. Copy the data using the shortcut "Ctrl + C" or "Cmd + C".
4. Paste the report in tab **"3. Paste Report Here"** of the eProtein Discovery Standalone Template (compatible with Microsoft Excel, not compatible with Google Sheet).
5. Review the data in the **"4. Output"** tab and ensure it is correctly labelled with corresponding constructs and cell-free blend labels.

6. The labelled data charts will be automatically plotted in the **"5. Charts"** tab ready for your review.

## Finishing the experiment

1. Remove the cartridge from the instrument drawer by lifting it as shown on the screen and place it in its original packaging. Press the **[Next]** button (Figure 31).

**Note:** there is no need to drain the base fluid out of the cartridge.


**Figure 31: Remove the cartridge from the instrument**

2. Remove the waste container from the holder, empty its content, and place it back on the instrument.
3. Remove the vial of base fluid and dispose of it with biohazard sharps waste container according to local waste disposal rules and regulations.

**Note:** Do not reuse consumed cartridges and dispose of any residual reagents, kits are intended as single use only.

4. Dispose the packaged used cartridge in a biohazard sharps container, according to local waste disposal rules and regulations.
5. The experiment report is available for download from the eProtein Discovery software.
6. Power down the instrument after use by pressing the [Power off] button (Figure 32)

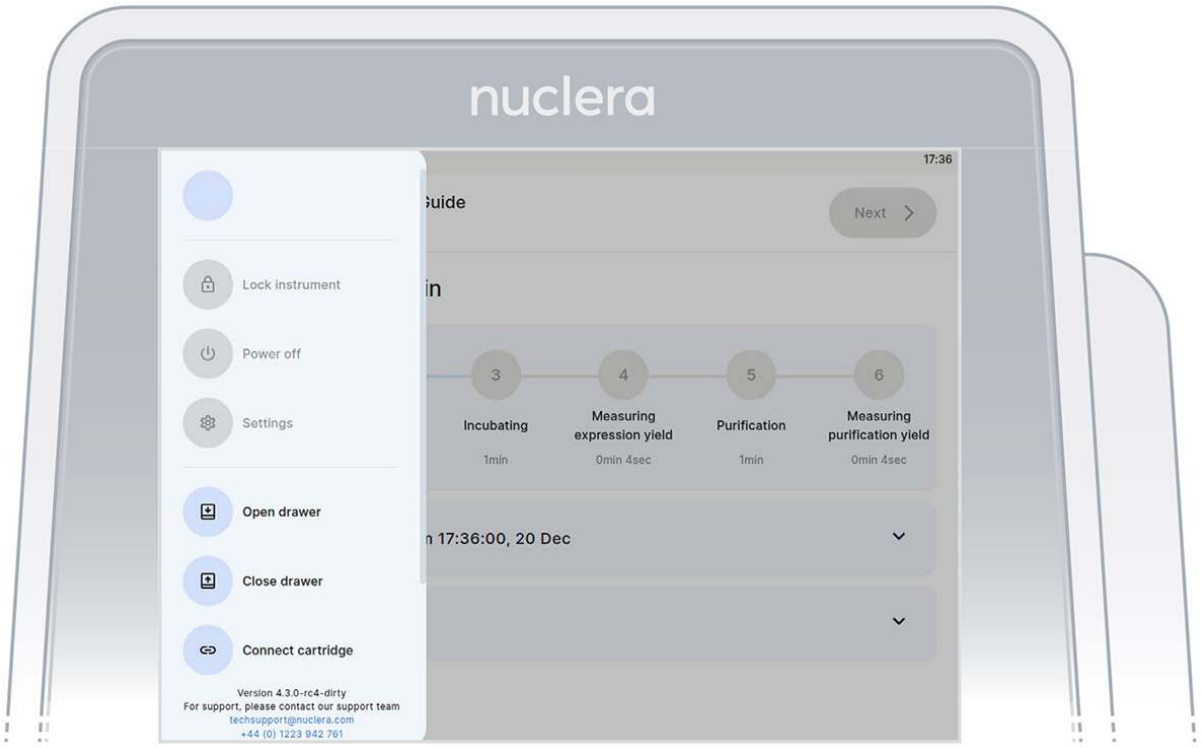



**Figure 32: Remove the cartridge from the instrument**

# Frequently Asked Questions (FAQ)

| Questions/ Issues | Answers |
|---|---|
| How do I contact the support team? | Email us at techsupport@nuclera.com Call us at +441223942 761 (UK phone number / WhatsApp Business) or +1 508-306-1297 (US phone number) |

| Questions/ Issues | Answers |
|---|---|
| Where can I suggest future improvements? | Please email us at techsupport@nuclera.com, your feedback is very important to us as it allows us to improve the instrument, the technology and our services. |
| A component or reagent is missing | If a component or a reagent is missing, please contact the Nuclera Technical Support team |

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UK Phone: +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

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**nuclera.com/legal for more info.**

# Scale-up Expression and Purification

## Scale-up expression and purification

📄 Download Page as PDF

### General information

The eProtein Discovery™ Scale-up protocol allows the in-tube protein expression of a specific eGene™ construct (DNA) using *E. coli* derived Cell-free Protein Synthesis (CFPS) reagents and additives.

The selection of the optimal eGene™ / Cell-Free Blend combination (eRecipe) is determined from the expression and purification screens performed on the eProtein Discovery instrument.

Depending on the protein of interest, the expected yield is as predicted from the experiment on the eProtein Discovery instrument.

The scale-up reaction volume in this guide is 200 µL. However, larger scale-up reactions, for example 1 mL, can also be achieved simply by adapting proportionally the volumes of the reagents and the Strep Beads.

The whole scale-up experiment takes less than 24 hours, as summarized in Table 1

| Protocol Step | Reagents and equipment required | Time |
|---|---|---|
| Expression reaction setup | • Cell Free Core Reagent<br>• Scale-up Additives (list in Table 2)<br>• 5 nM eGene™ construct (DNA) | 30 min |
| Protein Expression reaction | • Incubator, constant temperature of +29°C<br>• No agitation required | 15-18 hours (overnight) |
| Protein Purification | • Magnetic particle separator | |

The Scale-up kit NC3011 contains the Cell-free Core reagent, the purification Strep beads and the Wash and Elution buffers. The Scale-up Additives Kit NC3005 contains the additives listed in Table 2. The components of NC3011 and NC3005 are supplied in a Nuclera branded box with a grey stripe on the label.

| Additive name | Additive Description | Additive Characteristics |
|---|---|---|
| Additive Buffer | HEPES buffer pH 7.5 and surfactant | CFPS reaction buffer |

| Additive name | Additive Description | Additive Characteristics |
|---|---|---|
| PDI + GSSG Mix | Protein disulfide isomerase and oxidized glutathione | Chaperone and redox modification to oxidizing environment to support disulfide bond formation |
| TrxB1 | Thioredoxin reductase | Protects proteins from oxidative aggregation and inactivation and acts as a reductase in redox regulation |
| DnaK Mix | Chaperone DnaK mix | Chaperone mix to support folding and prevent aggregation |
| Zinc chloride | Zinc chloride solution | Cofactor that can be required for folding, stability, or activity |
| Calcium chloride | Calcium chloride solution | Cofactor that can be required for compaction, folding, stabilization, or activity |
| Manganese chloride | Manganese chloride solution | Cofactor for metalloenzymes for structure and activity |
| Cofactor Mix | Mix of NAD, acetyl-CoA,FAD, SAM, and PLP | Cofactors that assist in folding, stability and activity |

| Additive name | Additive Description | Additive Characteristics |
|---|---|---|
| GSSG | Oxidized glutathione | Redox modification to oxidizing environment |
| 3C protease | 3C protease solution | Protease to cleave off the N-terminal solubility tag at the specific amino-acid sequence |

## Scale up bundles content

### Scale-up kit (NC3011)

Strep beads and Scale-up reagents must be used within the expiration date stated on the kit box.

| Scale-up kit Strep Beads NC3011-1 | Cap color | Quantity | Storage instruction |
|---|---|---|---|
| Strep Beads | Orange | 5x400 µL | +4°C |

| Scale-up kit NC3011-2 | Cap color | Quantity | Storage instruction |
|---|---|---|---|
| Cell-free Core Reagent | Purple | 5x160 µL | -80°C |
| Wash Buffer | White | 5x3 mL | -80°C |

| Scale-up kit NC3011-2 | Cap color | Quantity | Storage instruction |
|---|---|---|---|
| Elution Buffer | Blue | 5x300 µL | -80°C |

### Scale-up additives (NC3005)

Scale-up additives must be used before the expiration date indicated on the box.

| Scale-up Additives NC3005 | Cap color | Quantity | Storage instruction |
|---|---|---|---|
| PDI/GSSG mix* | Green | 5x30 µL | -80°C |
| TRXB1* | Green | 5x30 µL | -80°C |
| DNAk mix* | Green | 5x30 µL | -80°C |
| Zinc chloride | Green | 1x150 µL | -80°C |
| Calcium chloride | Green | 1x150 µL | -80°C |
| Manganese chloride | Green | 1x150 µL | -80°C |
| Cofactor mix* | Green | 5x30 µL | -80°C |
| GSSG* | Green | 1x150 µL | -80°C |
| 3C protease* | Green | 5x30 µL | -80°C |

* Single use reagent that cannot be freeze/thawed multiple times

The image below shows how the additives are arranged in the box.


## User supplied reagents (not included in the kit)

- 5 nM eGene construct (DNA) generated using the Nuclera eGene Prep kit NC3009 or NC3008

## User supplied equipment

- Incubator (capable of maintaining a constant temperature of 29°C)
- Magnetic particle separator (compatible with 1.5 mL microcentrifuge

tubes)

- P1000, P200 pipettes and tips
- Vortexer
- Microcentrifuge
- 1.5 mL microcentrifuge tubes
- A tube rotator / agitator

# Scale-up expression and purification workflow

## Step 1: Cell-free Protein Synthesis (CFPS) reaction setup

1. Take the Cell-free Core Reagents aliquot, the two selected Scale-up Additives and the eGene construct from the freezer, and allow to thaw on ice. This will take approximately 5 minutes. Once thawed, keep the reagents on ice.
2. Centrifuge the Cell-free Core Reagent aliquot at 1000 rpm for 2 seconds and return to ice.
3. In a 1.5 mL microcentrifuge tube, set up the CFPS expression reaction according to Table 3

## CFPS expression

| Reagents | Volume | |
|---|---|---|
| Cell Free Reagent | 120 µL | 600 µL |

| Reagents | Volume | |
|---|---|---|
| Selected Additive 1 | 15 µL | 75µL |
| Selected Additive 2 | 15 µL | 75µL |
| 5 nM eGene DNA construct | 50 µL | 250µL |
| **Total reaction** | **200 µL** | **1 mL** |

**Table 3: CFPS expression reaction set-up, 200 µL or 1 mL.**

**Note:** it is recommended to run a 20 µL no-DNA negative control in parallel. In this case, substitute the 5 nM eGene construct with sterile water. Loading 3 µL of the negative control allows one to determine where the protein of interest is on the SDS-PAGE gel.

4. Vortex reaction tubes for 2 seconds to mix.
5. Centrifuge at 1000 rpm for 10 seconds.
6. Place samples in a tube rack and incubate the reaction mixture at 29°C overnight (15-18 hours) in a temperature controlled incubator.

**Note:** there is no requirement to agitate the samples during incubation.

## Step 2: Purification procedure

**Note:** the volumes and number of vials indicated in this procedure are for 200 µL CFPS reaction solutions.

**Note:** for optimal purification, it is recommended not to use CFPS reaction solutions larger than 500 µL. For example, a 1 mL CFPS reaction should be split into two 500 µL tubes before purification. Refer to the last column of

Table 4 for volumes used for a 1 mL CFPS reaction.

**Note:** the Wash Buffer and Elution Buffer contain a non-ionic detergent, to keep the purification conditions the same as on the eProtein cartridge. If the protein is required without detergents then please contact the Nuclera Technical Support team.

1. Take one vial of Wash Buffer (3 mL) and one vial of Elution Buffer (300 µL) from the freezer, and allow them to thaw at room temperature. This will take approximately 20 minutes.
2. Vortex buffers for 5 seconds to homogenize.
3. Take one vial of 400 µL Strep beads supplied in the kit from the fridge.
4. Prepare the Strep Purification Beads:
   a. Give the vial(s) of Strep Beads a quick spin in a microcentrifuge to pellet the beads
   b. Pipette up and down to fully resuspend the beads.
   c. Transfer the beads to a 1.5 mL microcentrifuge tube.
   d. Place the tube(s) of Strep Beads for 1 minute on a magnetic particle collector to pellet the beads.
   e. Aspirate the storage buffer supernatant and discard.
   f. Remove the tube from the collector and resuspend the Strep Bead pellet with 400 µL Wash Buffer by pipetting up and down repeatedly.
   g. Repeat steps c, d and e for a total of 2 washes.
   h. Pellet the Strep Beads on the magnetic particle collector, aspirate and discard the supernatant.
   i. Remove the tube from the collector and resuspend the Strep Bead pellet with 400 µL Wash Buffer to create a working solution ready to use (5% v/v).
5. Remove the 200 µL CFPS reaction tube prepared in step 1 from the 29°C incubator.
6. Give the CFPS a quick spin in a microcentrifuge.

7. Remove and reserve 3 µL of the CFPS reaction to run on a SDS-PAGE gel later (Label: Crude CFPS).
8. Pipette up and down three times the 400 µL Strep Bead suspension prepared in step 6 and place the tube on a magnetic particle collector for at least one minute to capture the beads.
9. Aspirate the supernatant and discard.
10. Remove the tube from the magnetic particle collector, centrifuge briefly to collect any residual liquid at the bottom of the tube, and then return the tube to the magnetic particle collector.
11. If there is any significant liquid remaining over the pellet, remove using a P10 pipette. **Note:** be careful to not remove any beads.
12. Pipette and transfer the CFPS reaction solution to the tube containing the Strep Beads.
13. Pipette up and down 10 times to resuspend the beads and incubate the suspension for 30 minutes at room temperature with agitation using a tube rotator or shaker at about 400 rpm. The beads should be suspended throughout the 30 min to ensure an optimal binding capacity.
14. After 30 minutes of incubation, place the tube on a magnetic particle separator and pellet the Strep Beads for 1 minute.
15. Aspirate and transfer supernatant to a new microcentrifuge tube. This supernatant contains all of the unbound, contaminating proteins from the CFPS reaction, along with any unpurifiable target protein. Retain the supernatant to run on an SDS-PAGE gel.
16. Remove the tube from the collector and resuspend the purification bead pellet in 400 µL of Wash Buffer. Pipette up and down 5 times to mix.
17. Place the tube on a magnetic particle separator and pellet the Strep Beads for 1 minute.
18. Aspirate the supernatant and discard. Retain the bead pellet and carry

forward to the next step.

19. Repeat twice steps 16-18 for a total of 3 washes.
20. Resuspend the beads in 250 µL Elution Buffer and place the tube on a tube rotator or shaker for 10 mins to elute the protein. Note: For proteins predicted to be expressed at 125 µg/mL or less, we recommend to use only 125 µL elution buffer.
21. Place the tube on a magnetic particle separator and pellet the Strep Beads for 60 seconds.
22. Aspirate and transfer supernatant into a new microcentrifuge tube (label aspirate: Purified). This tube contains the purified protein and can be stored for analysis and downstream applications.
23. Discard the Strep Bead pellet.

The expression and purification steps are summarized in Table 4.

| Component | Volume | |
|---|---|---|
| CFPS reaction | 200 µL | 1 mL |
| Prepared Strep Beads (5% v/v in Wash Buffer) | 400 µL | 2 x 1 mL* |
| Wash 1 | 400 µL | 2 x 1 mL* |
| Wash 2 | 400 µL | 2 x 1 mL* |
| Wash 3 | 400 µL | 2 x 1 mL* |
| Elution Buffer | 250 or 125 µL | 2 x 625* µL or 2 x 312.5* µL |

**Table 4:** Scale-up Kit purification summary. *When purifying CFPS reactions larger than 500 µL it is recommended to split the volume in two. Proportionally calculate the total volume required for the process and divide it into equivalent volume smaller or equal to 500 µL.

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**Nuclera Technical Support:**
UK Phone: +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Nuclera Customer Service**
UK phone: +44 (0) 1223 974 975
US phone: +1 508-306-1849
email: care@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

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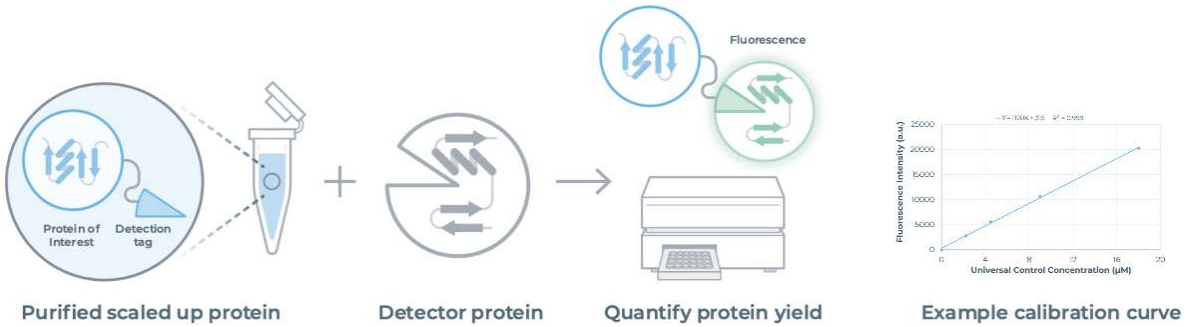
# eProtein Quantification Kit Protocol

## General Information

The Quantification Kit (NC3014) allows the user to accurately quantify protein yields immediately after scale up using the same fluorescence-based method used on-cartridge. Same system, same workflow, consistent results.




Compatible with standard plate readers, the detection tag on your scaled-up protein binds to a complementary detector protein, generating a fluorescent signal calibrated to a universal control.

## Features and benefits

**Scalable:** Consistent workflow, from screen to scale. Plate reader compatible
**Accurate:** Sensitive fluorescence-based detection ensures accuracy
**Streamilined:** Eliminates extra prep or method transfers. Scale up ready

# Contents

| Component | Volume | Cap Color | Storage Temperature | Tube Reference number |
|---|---|---|---|---|
| Detector Protein | 75 µL | Yellow | -80°C | NQK-01 |
| Universal Control | 20 µL | Yellow | -80°C | NQK-02 |
| Complementation Control | 20 µL | Yellow | -80°C | NQK-03 |
| Wash Buffer | 800 µL | White | -80°C | NQK-04 |

# User supplied equipment

▹ Microplate reader capable of measuring fluorescence at Ex/Em = 485/520.
▹ Fluorescence assay-compatible microplate (e.g. Corning, 3544) with the following specifications:

- 384-well
- Low volume (50 µL)
- Black walls with clear bottom
- Flat bottom
- Polystyrene with a nonbinding surface

▹ Adhesive PCR plate seal.

# Storage and Stability

▹ The kit must be stored at −80°C.
▹ Avoid freeze-thaw cycles.
▹ Thaw reagents on ice, briefly centrifuge, and pipette mix prior to use.
▹ Reactions should be assembled on ice.

# Reaction Assembly Overview

| | Standards | Negative Control | Positive Control | Protein Samples |
|---|---|---|---|---|
| Wash Buffer | 8 µL | 9 µL | 8 µL | 5 µL |
| Diluted Universal Control | 4 µL | X | X | X |
| Complementation Control | X | X | 1 µL | X |
| Protein Sample | X | X | X | 4 µL |
| Detector Protein | X | 3 µL | 3 µL | 3 µL |
| Total per Well | 12 µL | 12 µL | 12 µL | 12 µL |

## Standard Curve Preparation

1. Prepare the following serial dilution of the Universal Control.

- Prepare a fresh set of standards for each quantification.
- When performing serial dilution of the Universal Controls, pipette up and down 5 times to mix the reagents.
- Use a fresh pipette tip for each dilution step.
- Each dilution provides enough standard to set up triplicate readings.

| Standard Concentration | Volume of Universal Control | Volume of Wash Buffer | Dilution Number |
|---|---|---|---|
| 18 µM | 15 µL of undiluted | 15 µL | 1 |
| 9 µM | 15 µL of 18 µM dilution | 15 µL | 2 |
| 4.5 µM | 15 µL of 9 µM dilution | 15 µL | 3 |
| 2.25 µM | 15 µL of 4.5 µM dilution | 15 µL | 4 |
| 0 µM | 0 µL | 15 µL | 5 |

2. Add 8 µL of Wash Buffer to five wells of the 384 well assay plate in triplicate.
3. Add 4 µL of the above serial diluted standard in triplicate to the wells containing Wash Buffer (total well volume of 12 µL).

## Preparation of Negative Control Reactions

1. Add 9 µL of Wash Buffer in triplicate to the assay plate.
2. Add 3 µL of Detector Protein to the three wells containing Wash Buffer (total well volume of 12 µL).

## Preparation of Positive Control Reactions

1. Add 8 µL of Wash Buffer in triplicate to the assay plate.
2. Add 1 µL of Complementation Control to the wells containing Wash Buffer.
3. Add 3 µL of Detector Protein to the three wells containing Wash Buffer and Complementation Control (total well volume of 12 µL).

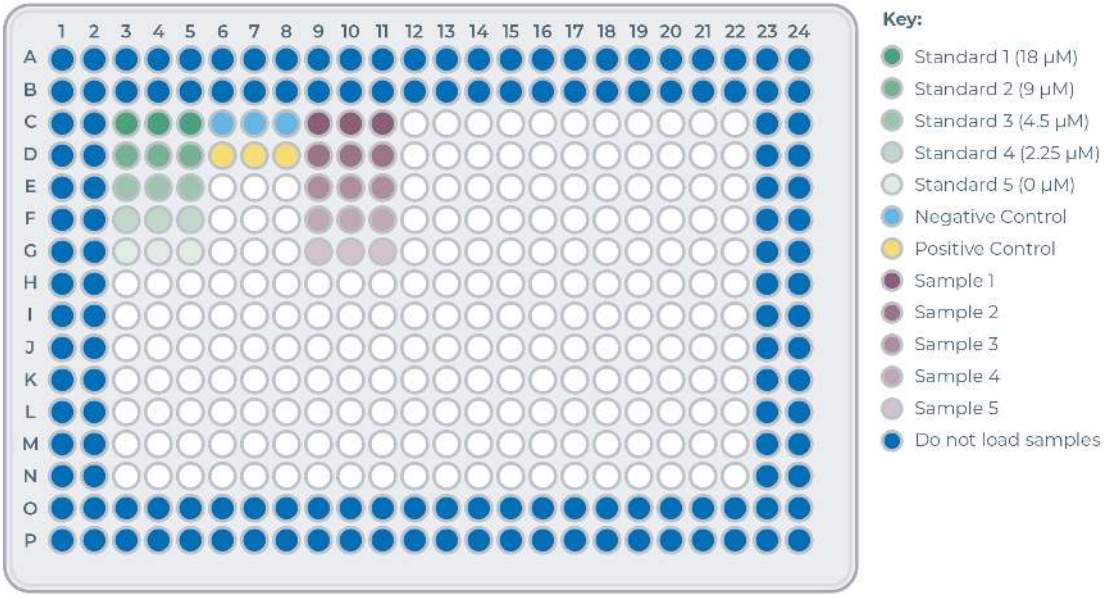
## Preparation of Test Samples

1. For each sample to be quantified, add 5 µL of Wash Buffer in triplicate to the assay plate.
2. Add 4 µL of sample protein to the wells containing Wash Buffer.

- Proteins with a predicted yield of more than 18 µM on the eProtein Discovery™ should be diluted 1 in 2 in Wash Buffer before quantification.

3. Add 3 µL of Detector Protein to each well containing Wash Buffer and sample protein (total well volume of 12 µL).

## Assay Plate Layout Overview




## Incubation

1. Seal the assay plate with an adhesive PCR plate seal. Ensure the plate is fully sealed before proceeding.
2. Briefly vortex the plate and pulse centrifuge for 15 secs.
3. Incubate the assay plate for 5 hours at 29°C.

- For overnight quantification, the assay plate can be incubated at 29°C directly in the plate reader with the program set to measure fluorescence after 5 hours. Ensure that the plate seal is left on and the program is set to bottom reading mode.

## Measurement

1. After 5 hours measure the fluorescence of all standards, controls, and samples in a plate reader with a 485 nm excitation filter and a 520 nm emission filter.

## Data Analysis

1. Calculate the average fluorescence reading for each standard.
2. Subtract the 0 µM reading from all other standard readings.
3. Plot the µM concentration of each standard against the measured fluorescence intensity, fit a linear trendline, and set the intercept to 0.
4. Calculate the average fluorescence intensity for each set of controls and protein samples.
5. Subtract the average fluorescence reading for the negative control from the positive control and protein sample measurements. The expected concentration of the positive control is approximately 9.0 µM.
6. Use the linear trendline equation to calculate the molarity of the protein samples and positive control.

- If the protein was diluted prior to reaction assembly (e.g. if the predicted concentration was above 18 µM), multiply by the dilution factor to calculate the concentration of the original sample.

**Nuclera Technical Support:**
UK Phone: +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Nuclera Customer Service**
UK phone: +44 (0) 1223 974 975
US phone: +1 508-306-1849
email: care@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

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# Guidelines to prepare SDS samples

## Prepare samples for SDS page gel

This section aims to give a guideline to run a commercial 15 combs SDS page gel.

For the SDS page gel you will need 4 tubes

| | Sample | Lab Grade Water | 3.6x Loading reducing dye |
|---|---|---|---|
| Eluted Protein | 3 µL | 5.7 µL | 3.3 µL |
| Core + NFW (negative control) | 3 µL | 19 µL | 9 µL |
| Crude CFPS | 3 µL | 19 µL | 9 µL |

**Standards**
To have a semiquantitative assay, you can prepare and run BSA standards alongside your samples.
Dilute your BSA sample to 1 mg/mL and make dilution as outlined in the table below to prepare your 3 standards (A, B and C)

| | Required Stocl | Lab Grade Water | 3.6x Loading reducing dye |
|---|---|---|---|
| Standard A | BSA 1 mg/mL | 24 µL | 176 µL |
| Standard B | Standard A | 50 µL | 150 µL |
| Standard C | Standard B | 50 µL | 150 µL |

**Load the SDS PAGE gel**

Load on the stain-free protein gel, 4-15% (15 well):

- 4 µL pre-stained protein ladder
- 4 µL eluted scaled-up protein sample
- 8 µL eluted scaled-up protein sample
- 4 µL No-DNA negative expression control
- 4 µL positive expression control
- 4 µL BSA standard A
- 4 µL BSA standard B
- 4 µL BSA standard C

Run at 200 V for 40 minutes

**Nuclera Technical Support:**
UK Phone +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
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One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

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# Protein Concentration & Buffer Exchange Guidelines

The Nuclera wash and elution buffers contain a nonionic detergent (12.5 kDa) required for effective cartridge droplet operations (Table 1). This is typically inert and well tolerated by proteins, but due to its unusually high molecular weight, can co-concentrate with proteins when using centrifugal concentrator devices, and care should be taken to use an appropriate filter MWCO to avoid problems associated with increasing the concentration of the detergent and the effect that could have on your protein of interest.

| Elution Buffer formulation at pH 8.0 | Wash Buffer formulation at pH 8.0 |
|---|---|
| • 0.1 M Tris-Cl<br>• 0.15 M NaCl<br>• 50 mM biotin<br>• 0.05% nonionic detergent | • 0.1 M Tris-Cl<br>• 0.15 M NaCl<br>• 0.05% nonionic detergent |

**Table 1: Formulations of the Elution and the Wash buffers supplied in the Scale-up kit.**

Detergents of this size (12.5 kDa) will be retained by filters with molecular weight cut-offs (MWCO) below 30 kDa, leading to unintended co-concentration with your protein. To prevent detergent retention and ensure optimal protein recovery, please follow these guidelines:

### ✅ Recommended Practice

- **For proteins > 60 kDa**: Use a **30 kDa MWCO concentrator**. This enables efficient separation of protein from detergents and minimizes loss of the target protein.
    - To reduce detergent content, dilute the eluted protein in a detergent-free elution buffer (Table 2) and reconcentrate using the same filter.
    - Note: Detergent removal may affect protein conformation, solubility, or alignment with screen predictions.
- **For proteins < 60 kDa**: Use a smaller MWCO filter and substitute purification buffers (wash and elution, Table 2) with your own detergent-free buffer with pH maintained between pH 7-8.
    - Be aware that buffer changes may affect protein folding and yield relative to cartridge predictions.

If you have any questions, please contact **Nuclera Technical Support**.

| Elution Buffer formulation at pH 8.0 | Wash Buffer formulation at pH 8.0 |
|---|---|
| • 0.1 M Tris-Cl<br>• 0.15 M NaCl<br>• 50 mM biotin | • 0.1 M Tris-Cl<br>• 0.15 M NaCl |

**Table 2: Formulations of the detergent-free Elution and the Wash buffers.**

### 🚫 Avoid

- Do not use concentrators with < 30 kDa MWCO when using buffers from the scale-up kit, as they will likely retain and concentrate detergents. This could cause problems with solution phase separation, and impact the quality of recovered concentrated protein.

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**Nuclera Technical Support:**
UK Phone +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

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Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

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# Primer design and general considerations for Adapt-PCR

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## Overview

In specific cases, the eProtein DiscoveryTM user may have the desired gene of interest (GOI) cloned into a vector backbone, or present on genomic DNA or linear templates. This protocol provides general primer design considerations to support users in converting these GOIs into eGenes compatible with the eProtein DiscoveryTM system.

## Design of loci-specific primers with overhangs

Prior to using plasmid or linear DNA as template for Adapt-PCR check for the presence of 3C or TEV protease cleavage sites in the sequence of the template. The presence of these sites should be avoided as their presence may interfere with Adapt-PCR and result in a low quality product. Amino acid sequences for 3C and TEV are listed below:

- 3C: LEVLFQGP

- TEV: ENLYFQS

Design loci-specific primers to amplify your GOI from the plasmid backbone. Primer design considerations are listed below:

- The 5’ end of the forward primer should NOT include the translation start codon (ATG) (see fig 1).
- The 5’ end of the reverse primer should NOT include the translation stop codons (TAA or TAG or TGA) (see fig 1).
- The loci-specific primers can be 18-25 bp long, ideally ending with a G or C or GC at the 3’ end.
- GC range of the loci-specific primers should be within 30% to 70%, with 50% to 55% being ideal.
    - The annealing temperature of the loci specific primers should be in the range of 55 to 70°C.
    - The annealing temperature of the loci-specific primer pair should not be higher than the extension temperature of the HF polymerase you wish to use.
- The loci-specific primer pair should not have a melting difference >5°C.
- Avoid self-complementarity within the primers and between the primer pair.

Append the loci-specific forward and reverse primers with the following tail sequences at the 5’ end of each of the primers:

- Forward Primer 5’ tail sequence: 5’ CTCGAGGTTCTGTTCCAAGGACCT
- Reverse Primer 5’ tail sequence: 5’ GCTCTGGAAGTACAGGTTCTC
- An example of loci-specific primers appended with the tail sequences is shown in Table 1.

Place the order of the oligos using standard desalting synthesis conditions.

Once the oligos are received, reconstitute it to standard 100 µM as per the manufacturer's guidelines using nuclease free water.

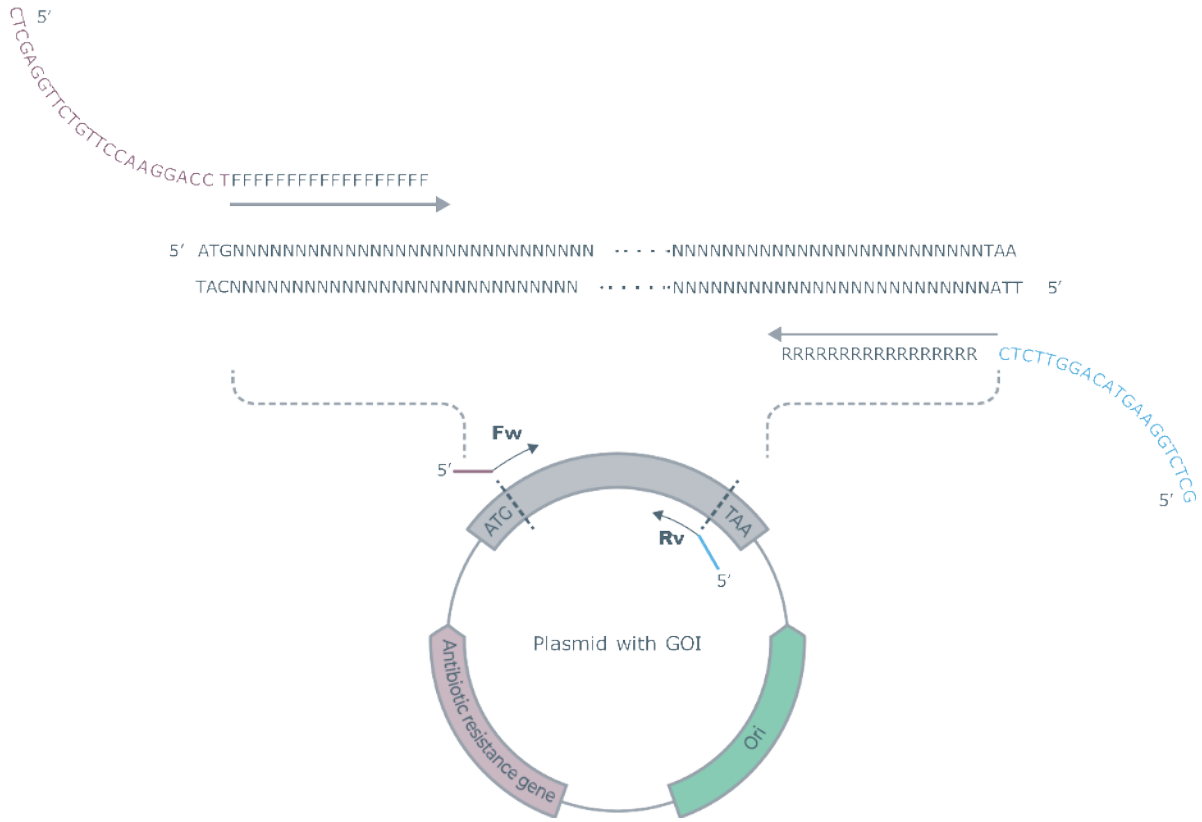


**Figure 1. Illustration indicating the positions of the forward and reverse primer pair to amplify the GOI from a plasmid backbone.**

| Primer | Tail sequence (5' to 3') | Loci Specific sequence (5' to 3') | C |
|---|---|---|---|
| Forward | CTCGAGGTTCTGTTCCAAGGACCT | FFFFFFFFFFFFFFFFF* | CTCGAGGTT |
| Reverse | GCTCTGGAAGTACAGGTTCTC | RRRRRRRRRRRRRRRR* | GCTCTGGAA |

**Table 1: An example of Adapt-PCR primer design based on the sequence illustrated in**

***F & R refer to the 5' to 3' forward and reverse primer sequences respectively.**

# General considerations for Adapt-PCR

Use a high fidelity PCR Mastermix. Carry out a low number of PCR cycles (up to 20 cycles).

- This step may require optimization to get a single band of the expected size. The typical yield from a single 50 µL PCR reaction is sufficient for subsequent steps. If using plasmid as the starting template, it is important to digest the template, as carried over plasmid DNA could interfere with CFPS reactions. Achieve this by adding Dpn1 enzyme directly to your PCR product prior to clean-up.
- Add 20 units of DpnI to 50 µL PCR product and incubate the reaction in a thermocycler set at 37°C for 1h.
- Prior to PCR purification, check the PCR product on an agarose gel. If you observe a single band of the expected size, proceed to purify the PCR product using any PCR cleanup method of your choice.
- Alternatively, resolve the bands on an agarose gel and proceed to excise the correct band to purify using gel extraction kit. To use the final product as template for eGene assembly application, normalize the PCR purified product to 2 nM using standard 10 mM Tris, pH 8.0 or 0.1X TE, pH 8.0.

# Risks and challenges associated with this protocol

Currently our eProtein Discovery platform is designed to express proteins codon optimized for expression in *E. coli*. Codon usage from other sources

such as humans, plants or even another genus of bacteria, with a different codon usage, may affect the total protein expression. Nuclera is unable to guarantee that an amplified construct will express. To gain maximum advantage of the eProtein Discovery platform, the User is strongly advised to codon optimize their GOI for expression in *E. coli*.

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**Nuclera Technical Support:**
UK Phone: +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Nuclera Customer Service**
UK phone: +44 (0) 1223 974 975
US phone: +1 508-306-1849
email: care@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

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**nuclera.com/legal for more info.**

# Assembly Guidelines to convert Linear eGene™ constructs into plasmids

This protocol provides general guidelines to clone linear eGene™ constructs into an expression vector of choice using Gibson Assembly® for use in E. coli scale up. The guidelines include primer design considerations supporting insert amplification, vector linearization, Gibson Assembly®, and transformation

## Reagents and Materials required

| Item | Item |
|---|---|
| Desired eGene™ construct | DpnI restriction endonuclease |
| Desired expression vector | PCR clean up kit |
| Appropriate primers to amplify eGene insert (see Guidelines) | Gibson Assembly® Master Mix (commercial or homemade) |
| Appropriate primers to amplify vector (see Guidelines) | DNA quantification tool (e.g. Nanodrop® or Qubit®) |

| Item | Item |
| --- | --- |
| Nuclease-free water | Competent cells (for transformation) |
| High-fidelity polymerase PCR master mix | LB agar plate with appropriate antibiotic |
| PCR tubes | LB medium |
| Thermocycler | Antibiotic appropriate for plasmid |

**Table 1: Material list.**

> ⚠ **HIGHLY RECOMMENDED**
>
> The transition from an eProtein Discovery™ screen to E. coli expression has only been validated using the pET vector series. Any alterations—such as changes to the promoter, removal of the lac operator/inducer, or differences in the ribosome binding site (RBS) or its spacing from the promoter may impact expression success.

# Guidelines

## Primer design

For Gibson Assembly®, consider the vector and insert as two fragments to be joined seamlessly into a circular plasmid. Therefore, two sets of primers (and two PCR reactions) will be required to amplify the expression vector and eGene™ selected. Below are guidelines for designing primers to

amplify the vector and eGene™ insert. See Figure 1 for an example with pET28a.

## Amplifying the Vector (Inverse PCR)

- Design the reverse primer to begin immediately upstream of ATG START codon (scan for the START codon just downstream of the Shine Dalgarno sequence/RBS). The 5’ end should not include CAT (reverse complement of ATG).
- Design forward primer to begin immediately after the TAA/TAG/TGA stop codon (just upstream of the terminator). The 5’ end should not include the stop codon.
- Primers should be:
- 18–30 bp in length.
- Annealing temperature should be between 55 and 70°C.
- Ensure Tm difference between primers is ≤ 5°C; both should anneal below the extension temperature of your polymerase.
- Design primers with 40–60% GC content (ideal: ~50–55%).
- Recommended: Use GC clamps (G/C at 3' ends), avoid secondary structures, and calculate Tm using tools like NEBuilder® or IDT OligoAnalyzer®.
- Avoid self-complementarity within the primers and between the primer pair.

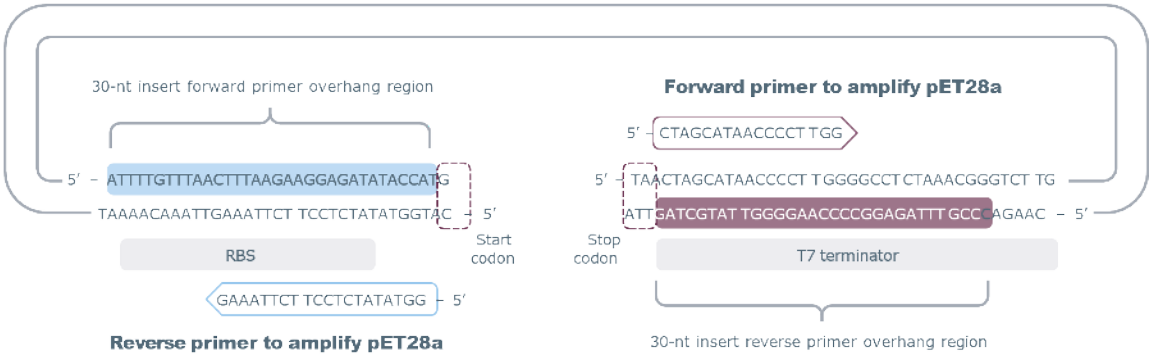
## Amplifying the eGene™ Insert

- Design forward and reverse primers with 30 bp 5' overhangs complementary to the ends of your linearized vector.
- Append the 30 bp overhang sequences to eGene™-specific primers using the loci-specific sequences provided in Table 2.

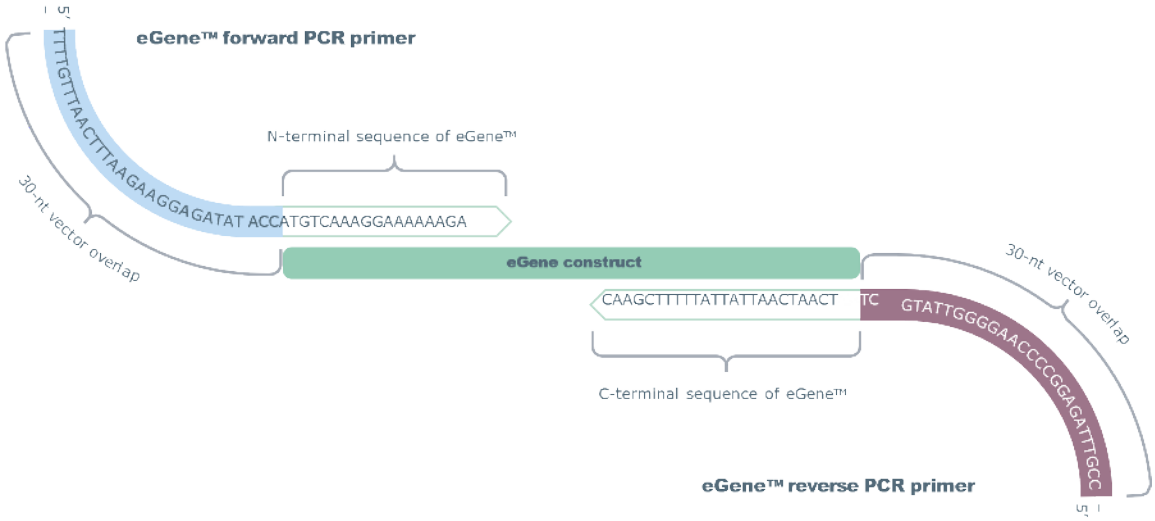
|Primer |Sequence (5’ to 3’)| |Forward |**[30 bp vector overhang]**ATGTCAAAGGAAAAAAGA| |Reverse |**[30 bp vector overhang]**TCAATCAATTATTATTTTTCGAAC|

**Table 2. Primer design guidelines for amplifying the eGene™ insert.**

**(A) pET28a fragment from PCR**




**(B) eGene™ fragment from PCR**




# General considerations for PCR

- Use a high-fidelity PCR Master Mix for both vector and insert amplification.

- Follow the manufacturer's guidelines for PCR Master Mix preparation and thermocycler settings.

- Amplifying the vector (Inverse PCR):

    - To prevent false positives during transformation, digest the original plasmid template using DpnI. After PCR amplification, add 20 units of DpnI per 50 µL of PCR product, and incubate at 37°C for 30 minutes. Heat-inactivate the enzyme by incubating at 80°C for 20 minutes before proceeding with clean-up.
    - Prior to PCR purification, check the PCR product on a 1% agarose gel. If you observe a single band of the expected size, proceed to purify the PCR product using any PCR cleanup method of your choice.
    - Alternatively, resolve the bands on an agarose gel and proceed to excise the correct band to purify using gel extraction kit.

- Amplifying the eGene™ Insert:

    - When amplifying the eGene™ insert the elongation time should take into consideration the full length of the eGene, including the gene of interest (GOI). See Table 3 for lengths.
    - Visualize the PCR product on a 1% agarose gel. If single bands are visible, proceed with PCR clean-up. If bands are faint or diffuse, use the band stab protocol from the eGene Prep guide. Do not use gel extraction for eGene™ inserts, as this may lower yields.
    - PCR clean up can be performed using any PCR cleanup method of your choice.

| eGene™ insert solubility tag variant | Size of insert |
|---|---|
| P17 | 386 bp + bp of GOI |
| CUSF | 554 bp + bp of GOI |
| FH8 | 491 bp + bp of GOI |
| TRX | 611 bp + bp of GOI |
| ZZ | 635 bp + bp of GOI |
| SUMO | 593 bp + bp of GOI |
| SNUT | 728 bp + bp of GOI |
| No solubility tag | 242 bp + bp of GOI |

**Table 3. Calculate the total size of the eGene™ insert for elongation time. GOI = gene of interest.**

# General considerations for Gibson Assembly® and transformation

- For Gibson Assembly® follow the manufacturer's instructions to assemble the insert and the linearized vector.
- It is recommended to transform the assembled product into competent E. coli (e.g., NEB® 5-alpha) and plate in LB agar plates containing the relevant antibiotic for the plasmid and incubate

overnight at 37°C.

- The linearized vector can be used as a transformation negative control. Ideally you should not see any colonies in this plate.
- (Optional) Select 5 random colonies, from the plates incubated overnight, to verify the presence of the insert by colony PCR (cPCR) using cPCR forward and reverse primer. cPCR forward and reverse primers can be found in Table 4.

|Primer |Sequence (5’ to 3’)| |Forward |ATGTCAAAGGAAAAAAGA| |Reverse |TCAATCAATTATTATTTTTCGAAC|

**Table 2. cPCR primer design to verify the presence of insert (optional).**

- Inoculate antibiotic supplemented medium relevant to the plasmid with 3-5 cPCR confirmed colonies for miniprep and subsequent sequence analysis.
- Transform sequence verified plasmids into an E. coli expression strain of choice.

## Disclaimers: NanoDrop and Qubit are registered trademarks of Thermo Fisher Scientific. IDT OligoAnalyzer is a registered trademark of IDT. Gibson Assembly is a registered trademark of Telesis Bio Inc.

## NEBuilder and NEB are registered trademarks of New England Biolabs.

**Nuclera Technical Support:**
UK Phone +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA: 1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

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# Circular eGene™ construct amplification for large-volume applications

After screening your Circular eGene™ constructs on the eProtein Discovery™ system, you may require more material than was originally supplied, particularly for large-scale cell-free protein synthesis (CFPS). This protocol outlines a standard approach to generating larger quantities through E. coli transformation, culture growth, and plasmid purification using a Midiprep kit. Following these guidelines will help produce more Circular eGene™ material, at the quality required, for downstream applications.

## Reagents and materials required

**Table 1. Materials list.**

| Item |
| --- |
| 1–10 ng of circular eGene™ construct |
| DH5-α competent cells (e.g. NEB® 5-alpha) |
| LB-Kanamycin plates (50 µg/mL Kanamycin) |

| Item |
| --- |
| Kanamycin stock solution (50 mg/mL) |
| LB media (100 mL) |
| PureLink™ HiPure Plasmid Midiprep Kit (Catalog number- K210004, ThermoScientific) |
| Isopropanol |
| 70% ethanol prepared in nuclease free water |
| Nuclease free water |
| Sterile bacterial culture tubes (14 mL size) |
| Sterile Baffled bacterial culture flask with filter cap (250 mL) |

# Equipment required

**Table 1. Equipment list.**

| Item |
| --- |
| +37 °C incubator and shaker |
| Microbiological safety cabinet |

| Item |
| --- |
| Cooling centrifuge with rotor for 15 and 50 mL centrifuge tubes and speed up to 20,000 g |

# Protocol

## *E. coli* transformation and culture preparation

1. Place a vial of competent E. coli DH5-α cells (e.g. NEB® 5-alpha) on ice until fully thawed (~5 min).
2. Add 1–10 ng of vendor-supplied circular eGene™ plasmid (not linearized) directly to the thawed cells.
3. Follow the manufacturer's instructions for transforming the cells.
4. Spread the transformation mixture onto LB agar plates containing 50 µg/mL kanamycin.
5. Incubate at +37 °C for 14–16 hours.
6. Pick a single, well-isolated colony and inoculate 2 mL LB broth containing 50 µg/mL kanamycin in a sterile 14 mL culture tube.
7. Incubate overnight at +37°C with shaking at 250 rpm.
8. Transfer 700 µL of the overnight culture into 70 mL LB broth containing 50 µg/mL kanamycin in a sterile 250 mL baffled flask.
9. Incubate overnight at +37°C with shaking at 250 rpm.
10. Transfer 50 mL of the culture into a 50 mL centrifuge tube.
11. Centrifuge at 4000 ×g for 10 min to pellet the cells.
12. Carefully discard the supernatant and invert the tube on absorbent paper to remove excess liquid.

13. Continue immediately with plasmid purification or store the cell pellet at –80°C until needed.

## Plasmid DNA purification

**Kit recommendation:** Use a plasmid Midiprep kit that produces nuclease- and endotoxin-free plasmid DNA (pDNA). We have tested and recommend: PureLink™ HiPure Plasmid Midiprep Kit (Thermo Scientific, Cat. No. K210004).

1. Follow the manufacturer's instructions for plasmid purification.
2. Elute the DNA in 5 mL of the kit-supplied elution buffer.

> **NOTE**
>
> DNA from the HiPure kit will be highly diluted. A precipitation step is required to concentrate the plasmid into nuclease-free water.

## Plasmid DNA precipitation and quantification

1. Add 3.5 mL of isopropanol to the eluted DNA. Mix well and incubate for 5 min at room temperature.
2. Centrifuge at 16,000 ×g for 30 min at +4°C.
3. A white DNA pellet should be visible at the bottom of the tube. Carefully remove the supernatant without disturbing the pellet.
4. Add 3 mL of freshly prepared 70% ethanol (in nuclease-free water). Invert the tube 4–5 times to wash the pellet.
5. Centrifuge at 16,000 ×g for 20 min at +4°C.
6. Carefully remove the supernatant. Ensure the pellet remains intact.
7. Briefly centrifuge again for 1 min and remove any remaining ethanol. **Important:** Residual ethanol will make it difficult to fully dissolve the

DNA.

8. Remove the cap and air-dry the pellet for 2–5 min until no liquid remains.
9. Add 50 µL nuclease-free water to the pellet.
10. Pipette up and down 10 times using a 100 µL pipette set to 50 µL.
11. Cap the tube, spin briefly, and transfer the DNA to a sterile, low-bind 1.5 mL microcentrifuge tube.
12. Measure the DNA concentration (260 nm). Expected yield: ~1000 ng/µL.

## DNA normalisation

1. Calculate molarity using the following formula to convert plasmid DNA concentration to molarity:

$$GOI(nM) = \frac{Concentration\ (ng/\mu L)}{[Length(bp) \times 617.96\ g/mol/bp] + 36.04\ g/mol} \times 10^6$$

2. Dilute the plasmid DNA to 100 nM using nuclease-free water.

## Adaptation to the Circular eGene™ Workflow

Follow the instructions in the Circular eGene™ Preparation Kit Guide to prepare constructs for Cell-free Scale-up, or proceed with *E. coli*-based Scale-up.



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**Nuclera Technical Support:**

UK Phone +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA: 1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

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# TEV Cleavage Protocol for Removal of C-terminal STREP and DET Tags

## Introduction

This protocol describes a TEV cleavage approach for removing the STREP and DET tags from the C-terminus of proteins produced using the eProtein™ Discovery Scale-up Expression and Purification guide. This protocol is designed to support users working with proteins where C-terminal tags may interfere with biological function, structural integrity, or downstream applications. By removing these tags through TEV protease cleavage, users can obtain a more native-like version of the protein for functional assays, binding studies, or structural characterization.

This protocol is designed to support users working with proteins where C-terminal tags may interfere with biological function, structural integrity, or downstream applications. By removing these tags through TEV protease cleavage, users can obtain a more native-like version of the protein for functional assays, binding studies, or structural characterization

**Note:** Following TEV cleavage, the protein will lack the STREP and DET tags for purification and quantification with the eProtein™ Quantification kit. The resulting protein will retain six non-native amino acids (ENLYFQ) from the TEV recognition sequence.

> **IMPORTANT**
>
> This protocol does not cover the removal of TEV protease. Please consult manufacturer guidelines if TEV protease removal is required for downstream applications.

# TEV cleavage

## Material Required

| Item |
| --- |
| Eluted protein scale-up |
| TEV protease stored in 1 mM TCEP (e.g. NEB® P8112S or MERCK® T4455) |
| SDS-PAGE equipment (for analysis) |

## Workflow Schematic

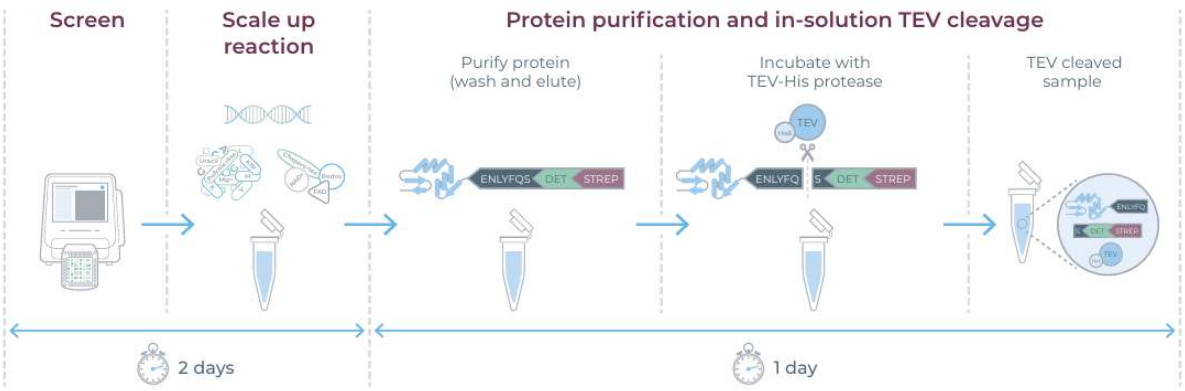

**Figure 1. TEV cleavage workflow schematic. The final sample will contain the cleaved protein, detached C-terminal DET and STREP tags, TEV protease, and potentially some uncleaved protein if cleavage efficiency is suboptimal.**

## Protocol

1. Express and purify the protein according to the standard eProtein™ Discovery Scale-up Expression and Purification guide.
2. Reserve 3 µL of the uncleaved sample for SDS-PAGE analysis.
3. Dilute TEV protease 1:20 ratio into the eluted protein. Example: Add 13 µL TEV to 250 µL of eluted protein. No additional buffers or reducing agents are required.
4. Gently pipette to mix and distribute the TEV protease.
5. Incubate at 4°C for 18 hours.
6. Optional: If required, remove TEV protease using the manufacturer's protocol.
7. Verify cleavage by SDS-PAGE against uncleaved samples from Step 2.

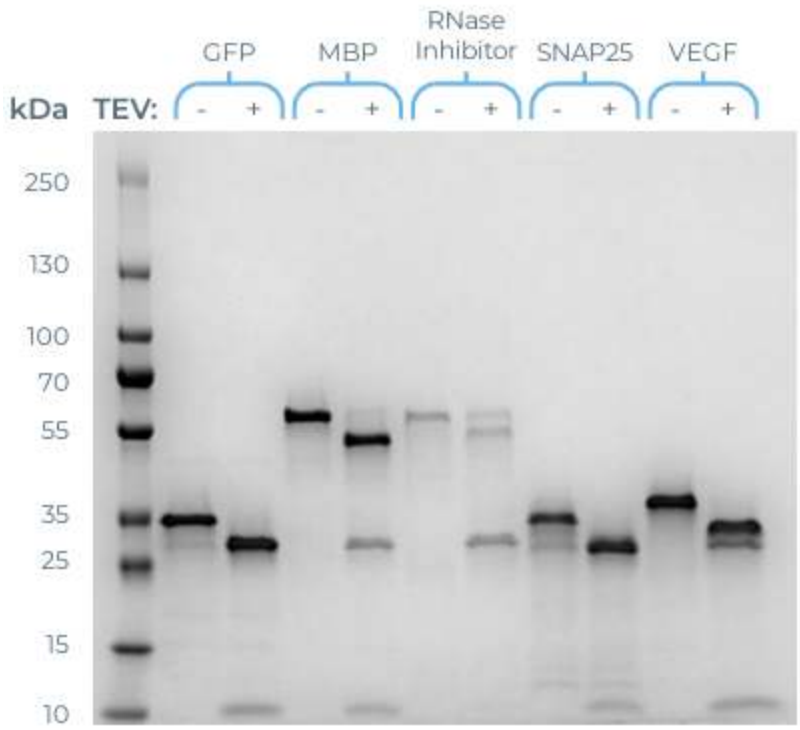


**Figure 2. Example SDS-PAGE of successful TEV cleavage. "–" indicates the uncleaved sample while "+" represents the TEV-cleaved products.**



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**Nuclera Technical Support:**
UK Phone +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**

Nuclera UK (HQ):

One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA: 1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

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# Biotinylating AviTag™ labelled proteins produced with eProtein Discovery™ using BirA

This protocol describes the process of biotinylating AviTag™-labelled proteins produced with eProtein Discovery™ using biotin ligase (BirA). The AviTag™ sequence must be introduced into the gene-of-interest, located between the 3C and TEV adaptor sequences.

## Reagents and Materials required

| Item | Supplier | Product Code |
|---|---|---|
| Elution Buffer (provided in Scale up kit) | Nuclera | NC3011 |
| BirA Biotin Ligase (recombinant, E. coli, ≥65%) | Merck | SRP0417-100UG |
| ATPSolution (100 mM) | ThermoScientific | R0441 |
| MgCl2 (0.2 M prepared in MilliQ Water) | - | - |

| Item | Supplier | Product Code |
|---|---|---|
| Zeba™SpinDesalting Columns, 40K MWCO | ThermoScientific | A57762 |

# Method

Aminimumproteinconcentration of 4 µM in elution buffer is recommended. If the protein concentration is lower, concentrate it using a spin concentrator with a molecular weight cutoff appropriate for the protein's size (not below 30 kDa).

## Prepare BirA

1. Prepare a 10 µMstock solution of BirA in elution buffer. Note: This can be stored aliquoted at-80 oC.
2. Dilute BirA to a working concentration that is 2.5X less than the concentration of the AviTag™-labelled protein.

## Biotinylating AviTagTM labelled proteins

1. Set up the biotinylation reaction in a 1.5 mL microcentrifuge tube according to Table 1.
2. ii. Incubate the reaction for 2 hours at 30°C.

| Item | Volume |
|---|---|
| Avi-Tag™ Protein (minimum concentration 4 µM) | 500 µL |

| Item | Volume |
|---|---|
| Diluted BirA (Concentration 2.5x less than protein) | 5 µL |
| $MgCl_2$ (0.2 M) | 15 µL |
| ATP (100 mM) | 15 µL |
| Elution Buffer | 65 µL |

**Table 1. Biotinylation Reaction Setup.** (Adjust volumes proportionally based on the protein volume while maintaining the same ratios).

## Removing excess biotin

Excess biotin is removed via buffer exchange using Zeba™ Spin Desalting Columns, 40K MWCO, following the manufacturer's instructions. For sample volumes between 300–800 µL, use product A57762 and the protocol below. For alternative sample volumes, choose an appropriate Zeba™ Spin Desalting Column and follow the manufacturer's guidelines.

1. Remove the column's bottom closure and loosen the cap (do not remove it completely).
2. Place the column in a collection tube and centrifuge at 700 x g for 2 minutes to remove the storage solution.
3. Discard the flowthrough and reposition the column in the collection tube.
4. Add 1 mL of the buffer of choice to the top of the resin and centrifuge tube at 700 x g for 2 minutes. Discard the flowthrough.
5. Repeat step 4 once.

6. Add 1 mL of the buffer of choice to the top of the resin and centrifuge tube at 700 x g for 3 minutes. Discard the flowthrough. Note: Ensure the resin appears white and free of liquid after each spin. If liquid is present, verify the centrifugation speed and time.
7. Transfer the column to a new collection tube.
8. Apply the biotinylated sample on top of the resin (optional) For low sample volumes or with low concentration apply a 40 µL stacker to the top of the resin bed after the sample has fully absorbed to ensure maximal protein recovery.
9. Sample recovery: Centrifuge at 700 x g for 3 minutes, collect the flowthrough containing the biotinylated sample, and discard the spin column. Additional Notes: Other spin columns have been tested and resulted in excess loss of protein. If preparing for Biacore SPR analysis, we recommend using Cytiva 1X HBS-P+ buffer.



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**Nuclera Technical Support:**
UK Phone +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA:
1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

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# Single Sign ON (SSO)

As an alternative to the standard email / password login, eProtein Discovery™ cloud-enabled system supports integration with external identity providers (IDPs) for single sign-on (SSO). In order to start using SSO, an identity provider (IDP) needs to be configured by the platform's Administrator. Supported types of identity providers:

- Google Workspace
- Microsoft Entra ID
- OpenID Connect v1.0

When an identity provider is configured and enabled, it appears on the login screen as a sign-in option. The configured identity provider must include the user's email, first name, and last name to be used for authentication.

SSOfig

## Configuring Organisation Identity Provider (IDP) - Administrator

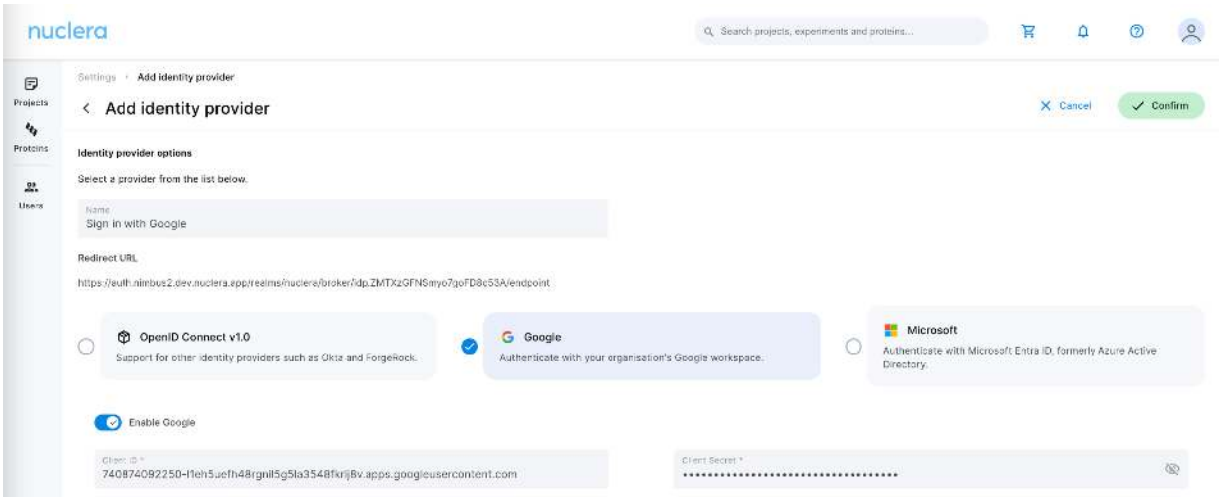
Access to IDP configuration is available to the platform Administrators in the "Settings" menu. To add new IDP integration, click the "New identity provider" button. The configuration parameters will vary depending on the type of IDP.

# Configuring Google IDP - Administrator

As a prerequisite, your organisation's IT department must set up OAuth client integration in the Google workspace and provide the eProtein Discovery™ platform

Administrator with the “Client ID” and “Client Secret”.

1. Enter a display name in the "Name" field. This name will appear on the corresponding button on the login screen.
2. Select type “Google“.
3. Enter the “Client ID“ and “Client Secret“.
4. Enable the IDP if you want it to be immediately available for login (can be enabled later).
5. Copy the “Redirect URL” and send it to your IT department to configure it as a valid redirect URL in your organisation's Google workspace.
6. Click “Confirm“.

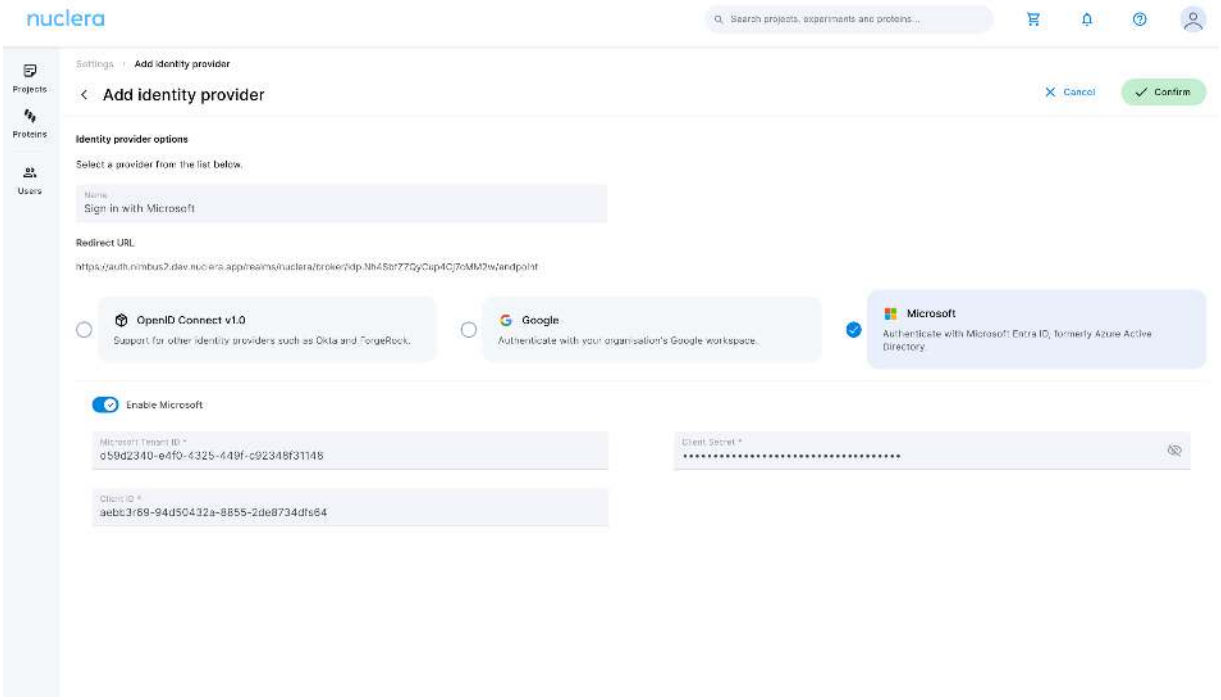


# Configuring Microsoft IDP - Administrator

As a prerequisite, your organisation's IT department must set up OAuth client integration in the Microsoft Entra ID workspace and provide the eProtein Discovery™ platform Administrator with the “Client ID”, “Client Secret”, and “Microsoft Tenant ID“. Microsoft Tenant ID must be specified as eProtein Discovery™ platform does not

support multi-tenant access for Microsoft accounts.

1. Enter a display name in the "Name" field. This name will appear on the corresponding button on the login screen.
2. Select type “Microsoft“.
3. Enter the “Client ID“, “Client Secret“, and “Microsoft Tenant ID“.
4. Enable the IDP if you want it to be immediately available for login (can be enabled later).
5. Copy the “Redirect URL” and send it to your IT department to configure it as a valid redirect URL in your organisation's Microsoft Entra ID.
6. Click “Confirm“.



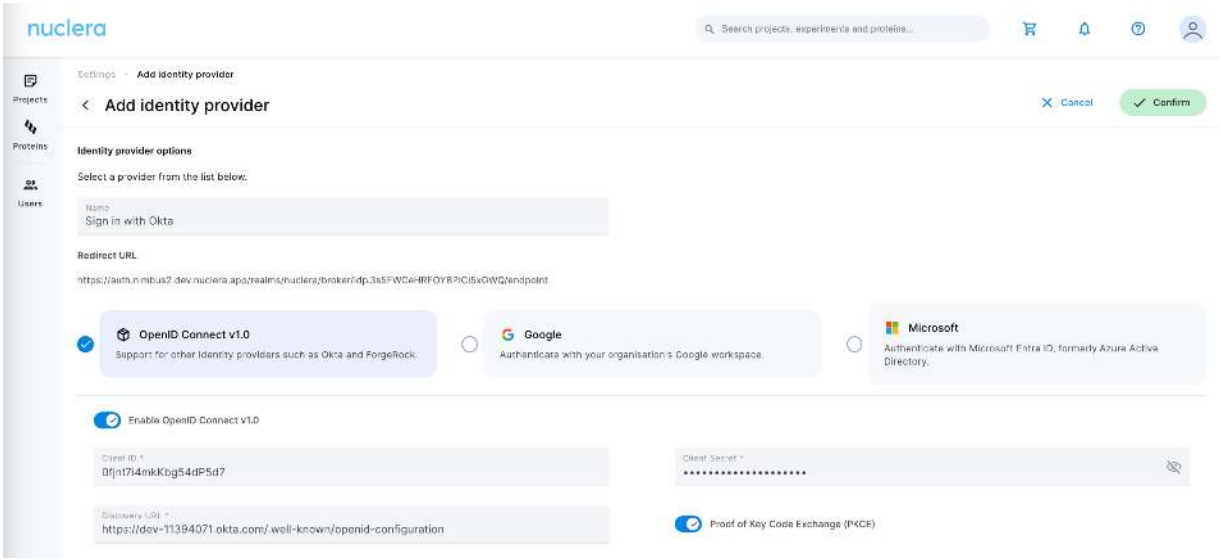

# Configuring OpenID Connect v1.0 IDP - Administrator

As a prerequisite, your organisation's IT department must confirm that your identity provider supports the OpenID Connect v1.0 protocol. "Client ID," "Client Secret," and "Discovery URL" need to be provided to the eProtein Discovery™ platform Administrator. Additionally, your organisation’s IT department should inform the Administrator if Proof Key for Code Exchange (PKCE) should be enabled for added security.

1. Enter a display name in the "Name" field. This name will appear on the corresponding button on the login screen.
2. Select type “OpenID Connect v1.0”.
3. Enter the “Client ID“, “Client Secret“, and “Discovery URL“.
4. Enable the IDP if you want it to be immediately available for login (can

be enabled later).

5. Enable / disable PKCE as recommended by the IT department.
6. Copy the “Redirect URL” and send it to your IT department to configure it as a valid redirect URL in your organisation's Google workspace.
7. Inform your IT department that the “Logout Endpoint URL” must be configured to “Redirect ULR“ + “/logout_response”. For example, if “Redirect URL” is https://auth.eu.nuclera.app/realms/organisation/broker/idp.xxx/endpoint, then the “Logout Endpoint URL“ must be https://auth.eu.nuclera.app/realms/organisation/broker/idp.xxx/endpoint/logout_resp onse.
8. Click “Confirm“.




# Inviting User Using SSO - Administrator

When using SSO, the user also must be invited by an Administrator. Without an invitation, the user will not be able to get access to the platform, even if previously configured IDP can authenticate this user successfully.

When inviting a user, the Administrator can check the “Invite using SSO“ box. The user will be added to the eProtein Discovery™ platform, a link to the platform’s login page will be sent to the user

in an email. Users will not be prompted to create a password, so the email and password login will not be available for this user.

# Using Sign Sign-On - Any user

If the corporate identity provider has been set up by an Administrator, the user can start using it by clicking a button on the login screen. If a user has already been invited and previously used an email and password to log in, this type of login will continue to work. In this case it is also possible to switch to using the SSO login method only. To do this, “SSO only access“ can be enabled in the “Edit Profile“ menu. To enable “SSO only access“, existing user’s sessions must be authenticated using SSO. If SSO is used, first name and last name cannot be changed in the “Edit Profile“ menu, as they are obtained directly from the configured organisation identity provider. To bring back email and password authentication, “SSO only access” can be disabled. In this case an email with a link to set up a new password will be sent to the user. Until “SSO only access“ is switched off, “Reset Password” will not be available for the non-Administrators.

# Frequently Asked Questions (FAQ)

## General

1. I want to migrate users of my organisation to use single sign-on (SSO) instead of email and password.

- As an Administrator, you must configure an identity provider and ensure that it is working correctly. After that, each user can enable “SSO only access“ in the “Edit Profile”.

2. I want to migrate users of my organisation to use email and password instead of single sign-on (SSO).

- As an Administrator, go to the "Manage Users" menu and request a password reset for all users in your organisation who have "SSO only access." This will allow them to set a new password. For these users "SSO only access" will be disabled.

3. In my organisation, SSO is used. I need to invite external collaborators who cannot be authenticated by our identity provider.

- External users can be invited to authenticate using an email and password. The administrator must ensure that "Invite using SSO" is unchecked when sending the invitation.

4. I’m getting “409 Registration incomplete” when using SSO.

- This error can occur if your organisation’s identity provider does not contain your user’s email, first name, and last name. Make sure that you have this information specified in your identity provider. If it does not help, contact Nuclera Technical Support team.

## Technical issues (software / hardware)

1. The user forgot the password.

- Users can reset their password from the login screen by clicking the "Forgot your password?" button. However, if a user has enabled "SSO only access" or was invited via SSO, the password cannot be reset. Administrators can request a password reset from the "Manage Users" menu, which is accessible to platform administrators.

2. The administrator enabled "SSO only access" but lost access to the eProtein DiscoveryTM cloud-enabled platform due to a misconfigured SSO identity provider.

- Contact the Nuclera Technical Support team (techsupport@nuclera.com)

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US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA: 1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

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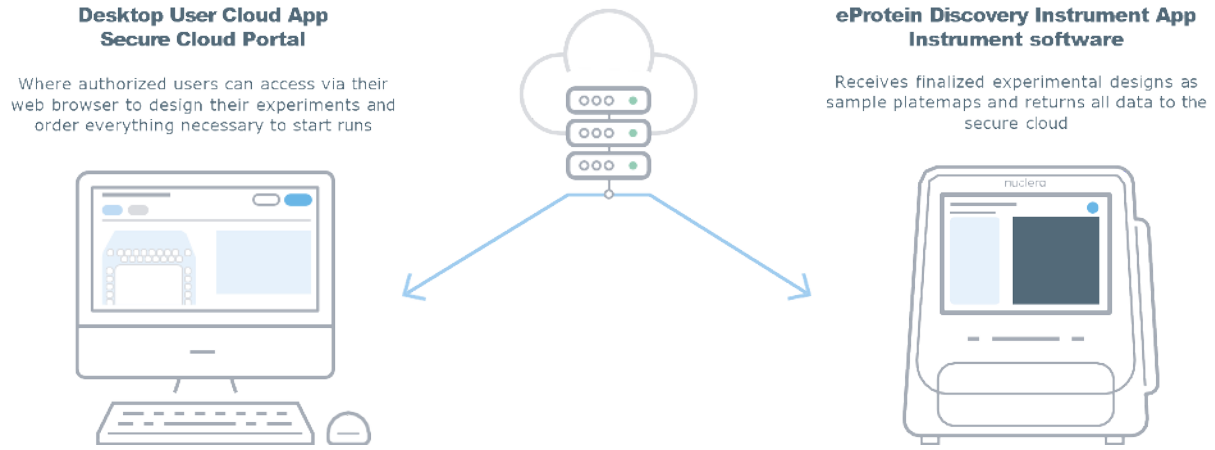
# Cloud Connectivity and Setup guide

## Cloud-connected Instrument software summary, security

overview and connection requirements Nuclera's eProtein Discovery™ cloud-connected instrument is the only end-to-end protein screening and optimization system that accelerates protein design, cell-free expression, purification and solubility characterization. For the best experience with the eProtein Discovery™ instrument, it needs to be connected to the internet so users can log in to their secure eProtein Discovery™ web space, set up their experiments, order necessary DNA and other consumables and then, once the experiment is loaded and started, monitor its progress and review the results remotely from the comfort of their office using just the web browser. Nuclera is passionate about protecting its customers' information both in the cloud and on its benchtop instrument. This document provides an overview of Nuclera's Instrument and cloud connection requirements.

## Software communication topology and security overview

Nuclera's eProtein Discovery Platform has two major software components; a cloud portal and instrument embedded software, which communicate with each other through a secure, encrypted channel.




## Cloud software overview

The eProtein Discovery cloud portal software is provided as a web based service from Nuclera's virtual private cloud. Each customer is provided with their own secure data space where user login access is required and gated by their organization. When a new space is set up for a customer, they nominate at least one user in their organization as an admin user with full control of the space. Organization admin users can then invite other users from their organization and give them access to individual projects. Projects are where proteins (with their sequences and other metadata), experiments and instrument run data are stored and accessed by users authorized by their organization admins.


When users are invited by their organization admins, they receive an invitation email with a temporary registration link. This link allows them to register their account and set a password in accordance with their organization's policies, which can be changed later. Nuclera also enforces a minimum 12 character password length for eProtein cloud accounts. Users who are required to operate the benchtop instrument must also set up an instrument access PIN.

From cloud software version 1.2 onwards, users can also utilize their organization's identity provider to allow them to log in with Single Sign On (SSO). Nuclera recommends this option if customers require multi factor authentication or that their login credentials adhere strictly to their own organization's IT requirements.

Once signed up, users can start registering their proteins of interest by submitting DNA or amino acid sequences inside the project(s) they are members of. The compatibility of these sequences with DNA and protein synthesis will be checked automatically by the cloud software. They can then order DNA through the software, and design their experiments which they will then be able to run on their eProtein discovery benchtop instruments.

Nuclera's eProtein Discovery cloud software is built with the best security practices in mind. Customer data, such as DNA sequences are only accessible by the authorized users and Nuclera representatives who fulfill these orders and provide technical support (if and when required). The data is encrypted at rest with AES-256 encryption. Data in transit to and from the user browser is encrypted by TLS. Nuclera's instrument platform's communications with Nuclera's cloud are encrypted using transport layer security version three (TLS 1.3) and WireGuard protocols

## Instrument embedded software overview

Nuclera's eProtein Discovery instrument has an integrated onboard computer (NVIDIA) loaded with Nuclera's software on a custom linux-based operating system. The instrument software controls all operations on the instrument and cartridge. Experiments designed on the cloud software will automatically be transferred to the instrument software where they are accessible through the intuitive touch screen located at the front of the instrument. Users can follow step-by-step instructions on the interface to load and run their experiments. The instrument software carries out the experiment, records the results and uploads result files. A secure network channel between the instrument and cloud-based software enables users to monitor the experiment in real time and analyze data upon completion. Communication is performed via a custom API and secured with TLS 1.3. Users will need to enter their PINs to access the instrument and run their experiments.

eProtein Discovery instruments are able to receive critical software and firmware updates via the same network channel from Nuclera.

# eProtein Discovery instrument connectivity requirements

Nuclera's instrument is capable of functioning in several connectivity modes:

- **Cloud connected depending on the customer side network configuration in either:**
  - Fully cloud connected mode: The instrument has an internet connection where it is capable of reaching Nuclera's cloud systems for experiment synchronization, system metrics and remote support.
  - Cloud connected without remote support mode: The instrument has an internet connection and is able to connect to Nuclera's cloud systems for experiment synchronization and system metrics but remote support is disabled.
- **Standalone depending on the customer-side network configuration in either:**
  - Standalone mode: Nuclera's instrument is fully disconnected from the network, experiments are designed right on the instrument touch screen. Data can be exported by USB flash drive.
  - Standalone + data export connection mode: Nuclera's instrument is connected to a customer controlled network with some limited connectivity to allow data export. Experiments are designed right on the instrument touch screen. The instrument is capable of connecting to a local network share to export the data from the experiments

In order for the cloud-enabled instrument to perform the above mentioned functions it needs to be connected to the internet via an ethernet cable. It is

configured to automatically receive its IP address, gateway and other network settings via dynamic host configuration protocol (DHCP). In case the customer's local IT setup requires a static IP address configuration, this can be set up either by the end user from the instrument touch screen (from version 5.1 onwards) or by Nuclera's field application specialist at the point of installation. Nuclera's instrument can also support access to the internet through an https proxy if required (from version 5.1 onwards) however it will not tolerate secure sockets layer (SSL) termination occurring on inline network devices, e.g. for the purpose of deep packet inspection.

Nuclera's instrument will be shipped to clients in standalone mode and can be reconfigured remotely for connected operation assuming that the remote support services have been allowed by the customer's IT team. Alternatively, the instrument can be reconfigured to operate in cloud connected mode without remote support during the installation visit and assuming that there is a suitable WiFi or cellular signal that Nuclera's field application specialist can connect to on a temporary basis to configure the instrument.

The instrument connects to Nuclera's virtual private cloud, hosted on Google Cloud Platform (GCP). The instrument uses Google Cloud Storage for experiment data storage. Google does not provide a fixed IP address range for its cloud storage offering. Consequently, due to various cloud services (including GCP) not using fixed IP addresses, we cannot guarantee that the instrument will behave correctly if connections are limited using an IP address allow list.

When connecting to a network share, the shared folder can be hosted either on a network attached storage device or a locally supplied laptop. Nuclera recommends that customers use a network attached storage device or other Samba3 compatible network share. The customer must ensure that the correct network ports have been opened in any intervening

firewalls to allow the instrument to connect to the network share, including in the Windows firewall if connecting to a locally supplied laptop.

Nuclera's instrument relies heavily on having the correct time. Where a customer's network has an available DHCP server, if the DHCP server specifies a set of network time protocol (NTP) servers, these will be used. For customer networks where a static IP address is used or where DHCP is not available, the instrument needs to be able to connect to ntp.ubuntu.com as per the table below. During initial setup, Nuclera's instrument will require additional connectivity which can be removed subsequently, without affecting normal operation.

In case the customer needs to set up firewall rules based on a source IP address or address range for Nuclera's instrument(s), Nuclera strongly recommends that customers make use of DHCP reservations to ensure that the same IP address is assigned to a given instrument following a reboot. While the instrument is capable of supporting a static IP address configuration (from version 5.1 onwards) combining static IP configuration of the instrument with a DHCP enabled network is strongly discouraged as an IP address conflict may arise when changes are made in future, resulting in an impact to the customer's network.

## Limiting the instrument connection

For best results, Nuclera recommends customers use the instrument in fully cloud connected mode (including remote support). This is best achieved by connecting the instrument to a network which allows internet access without any filtering or proxies but in case this is not possible the following set of URLs will allow the instrument to connect in a more limited way.

## Installation and normal operation

| Destination hostname | Destination port | Protocol | Purpose |
|---|---|---|---|
| auth.eu.nuclera.app | 443 | TCP | Initial installation |
| auth.us.nuclera.app | 443 | TCP | Initial installation |
| downloads.mender.io | 443 | TCP | Initial installation |
| esm.ubuntu.com | 443 | TCP | Initial installation |
| eu.hosted.mender.io | 443 | TCP | Initial installation |
| packages.wazuh.com | 443 | TCP | Initial installation |
| pkgs.tailscale.com | 443 | TCP | Initial installation |
| repo.download.nvidia.com | 443 | TCP | Initial installation |

| Destination hostname | Destination port | Protocol | Purpose |
|---|---|---|---|
| superusers.eu.nuclera.app | 443 | TCP | Initial installation |
| superusers.us.nuclera.app | 443 | TCP | Initial installation |
| ppa.launchpad.net | 443 | TCP | Initial installation |
| repo.netdata.cloud | 443 | TCP | Initial installation |
| api.netdata.cloud | 443 | TCP | Normal operation |
| app.netdata.cloud | 443 | TCP | Normal operation |
| instruments.eu.nuclera.app | 443 | TCP | Normal operation |
| instruments.us.nuclera.app | 443 | TCP | Normal operation |
| mqtt.netdata.cloud | 443 | TCP | Normal operation |
| storage.googleapis.com | 443 | TCP | Normal |

| Destination hostname | Destination port | Protocol | Purpose |
|---|---|---|---|
| | | | operation |
| us-east1-netdata-analytics-bi.cloudfunctions.net | 443 | TCP | Normal operation |
| ntp.ubuntu.com | 123 | UDP | Normal operation |
| s15f98bady02.cloud.wazuh.com | 443,1514,1515 | TCP | Normal operation |

**Remote support and network data export**

| Destination hostname | Destination port | Protocol | Purpose |
|---|---|---|---|
| derp1-all.tailscale.com | 443 | TCP | Remote support |
| derp10-all.tailscale.com | 443 | TCP | Remote support |
| derp12-all.tailscale.com | 443 | TCP | Remote support |
| derp18-all.tailscale.com | 443 | TCP | Remote |

| Destination hostname | Destination port | Protocol | Purpose |
| --- | --- | --- | --- |
| | | | support |
| derp19-all.tailscale.com | 443 | TCP | Remote support |
| derp8-all.tailscale.com | 443 | TCP | Remote support |
| derp1-all.tailscale.com | 3478 | UDP | Remote support |
| derp10-all.tailscale.com | 3478 | UDP | Remote support |
| derp12-all.tailscale.com | 3478 | UDP | Remote support |
| derp18-all.tailscale.com | 3478 | UDP | Remote support |
| derp19-all.tailscale.com | 3478 | UDP | Remote support |
| derp8-all.tailscale.com | 3478 | UDP | Remote support |
| controlplane.tailscale.com | 443 | TCP | Remote support |

| Destination hostname | Destination port | Protocol | Purpose |
|---|---|---|---|
| log.tailscale.io | 443 | TCP | Remote support |
| log.tailscale.com | 443 | TCP | Remote support |
| Customer NAS / file server | 139,445 | TCP | Network data export |

## Typical IT services Q&A

**Why do users require access to the eProtein Discovery cloud software?**
Users require access to the cloud software to:

- Check their protein sequences compatibility with eProtein Discovery DNA synthesis and cell-free protein synthesis
- Order DNA templates for their proteins from Nuclera
- Design their experiments
- Securely push designed experiments to the instrument via internet connection
- Monitor experiments progress
- Review and analyze the results

**What kind of sensitive data is stored in eProtein Discovery cloud software and why?**

Protein sequence data (that is considered the most sensitive data that is entered by users) is stored securely by the eProtein Discovery cloud software within the organization space in order to:

- Automatically check for compatibility with DNA synthesis and cell-free protein synthesis
- Synthesize DNA molecules and deliver to your lab for your experiments.

With other DNA providers these sequences are typically communicated by email to order the DNA, and then linger in their inbox. We take the security of your sequences (as well as all other data stored in our cloud system) to the next level by storing it in an encrypted space which is only accessible by your authorized users, or by our employees who need to fulfill your DNA synthesis order or provide technical support.

**How is the data online protected?**
The protein sequences, experiment results and all other data is encrypted at rest with AES-256 encryption and are only accessible by authorized users

**What kind of device is the cloud-enabled eProtein Discovery benchtop instrument?**
The cloud-enabled eProtein Discovery benchtop instrument is an IoT device with embedded computer (NVIDIA) with Nuclera's custom linux-based OS and proprietary software which runs the instrument and communicates with the eProtein Discovery cloud software.

**How does the eProtein Discovery benchtop instrument communicate with the cloud?**
The eProtein Discovery benchtop instrument communicates with the eProtein Discovery cloud software through a TLS encrypted API. This connection is one way, the cloud software does not try to connect to the instrument.

**How would the instrument receive software updates?**
Updates are received over the air, the eProtein Discovery benchtop

instrument software and firmware updates will be offered to customers by Nuclera when available and required. The instrument needs to be online to receive an update.

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**Nuclera Technical Support:**
UK Phone +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

**Offices:**
Nuclera UK (HQ):
One Vision Park, Station Road, Cambridge, CB24 9NP, UK

Nuclera USA: 1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

**www.nuclera.com**

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# eProtein Discovery™ Standalone System Setup Guide

## Data export setup guidelines

The eProtein Discovery instrument in Standalone mode has no internet connectivity to receive or send instrument run data to the partnered cloud software. Therefore a Standalone mode requires an alternative method to export experiment data from the instrument to be analyzed on a Windows PC/laptop on MS Excel.

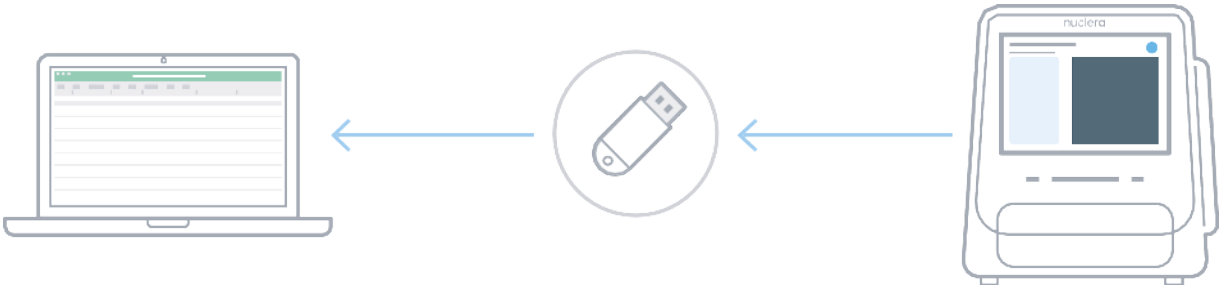
Data export can be enabled using three primary options:

1. Export to a USB flash drive
2. Export to a NAS drive on a local-area network
3. Export to a shared folder on an attached laptop

These are described in further detail on the following pages, along with a list of equipment that you will need to provide to facilitate each option.

Please share this document with your IT department to choose the appropriate option and prepare the required equipment before installation.

# Export to a USB flash drive




In this method experiment data is retrieved by inserting a USB flash drive into the instrument and exporting the data onto it, then inserting it into a Windows laptop and using an Excel spreadsheet to analyze the data. Please check that your organization's IT security policies allow USB flash drives before choosing this option.

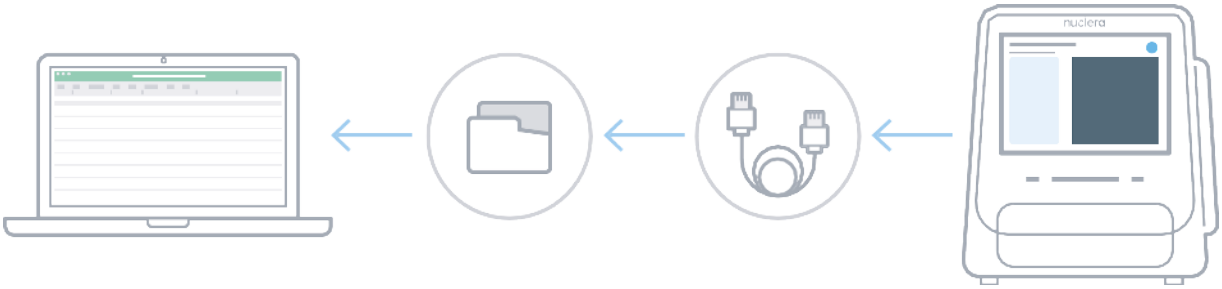
## Equipment to be provided by the customer during installation:

**1. A USB flash drive**

- Must have at least 2 GB of disk space (16 GB recommended)
- Only non-encrypted USB drives are currently supported
- Must be formatted with one of the following filesystem types:
  - exFAT (recommended)
  - EXT4
  - FAT32
  - NTFS

2. A Windows laptop with a USB port and Microsoft Excel

# Export to a NAS drive on a local-area network




If your organization has a NAS (network attached storage) drive on the local area network then the instrument can be connected to the network and experiment data exported to the NAS drive, where it can then be read by a Windows laptop connected to the same network to analyze the data using an Excel spreadsheet.

To use this option, there must be an Ethernet port in the lab where the instrument will be situated so that it can be connected to the LAN. During installation you will need to provide the following information:

- The IP address of the NAS drive
- The name of the shared folder
- The username and password of a user who has write access to the shared folder

Please check with your IT team that an appropriate Ethernet port is available in the lab and that any network or firewall configuration to allow the instrument to connect to the LAN and NAS drive has been performed prior to installation. Contact support if you need any of the following:

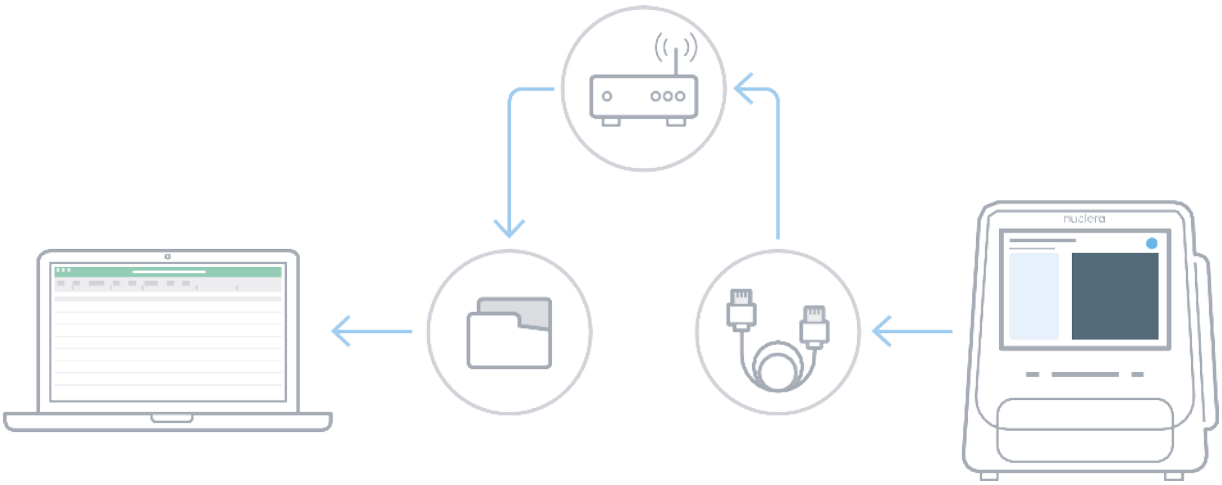
- The MAC address of the instrument

- The instrument to be configured to use a static IP address

## Equipment to be provided by the customer during installation:

1. A LAN port in the lab that the instrument can be connected to
2. A NAS drive on the local area network
    - The NAS drive must support the SMB v3 protocol
3. A Windows laptop with Microsoft Excel connected to the same network with access to the shared drive

# Export to a shared folder on an attached laptop




If you have a Windows laptop in the same lab as the instrument then it can be connected to the instrument using an Ethernet cable and router to allow the instrument to export experiment data to it. The experiment data can then be analyzed on the laptop using an Excel spreadsheet.

The Windows laptop must have a shared folder that is accessible from the network. During installation you will need to provide the following

information:

- The name of the shared folder
- The username and password of a user on the laptop who has write access to the shared folder

Please ask your IT team to set up the shared folder (we suggest "C:\eProteinReports") and user with write access, and to configure any security settings to allow the folder to be visible to other machines on the network prior to installation. In particular please ask them to ensure that:

- "File and printer sharing" is enabled for both public and private networks (this is necessary because Windows considers some direct connections to be "public")
- Any firewall running on the laptop has been configured with appropriate rules to allow the folder to be visible on the network (e.g. unblocking the file sharing port TCP 445)

The laptop setup can be verified prior to installation by connecting this and another laptop to the same LAN and then checking that the other laptop can see the shared folder and copy files to it using the username and password of the user with write access.

Equipment to be provided by the customer during installation:

1. A Windows 10 or later laptop near where the instrument will be installed in the lab

- Must have a spare Ethernet port, or a spare USB port for use with a USB-to-Ethernet dongle
- Must be configured with a network-accessible shared folder and user with write access as described above
- Nuclera Field Application Specialists will need temporary admin access

to this laptop during installation

Nuclera will provide the router, Ethernet cables and dongles required to connect the laptop to the instrument.

> **NOTE**
>
> We are committed to providing pre-installation assistance and support to ensure a smooth and successful installation process. If you have any questions or concerns, please don't hesitate to reach out to us.

| **Nuclera Technical Support:** | |
|---|---|
| Email | techsupport@nuclera.com |
| UK Phone | +44 1223 942 761 |
| US Phone | +1 508-306-1297 |

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# Videos

See our range of videos

### AlphaFold

Seamless integration of AlphaFold into Nuclera's eProtein Discovery™ Cloud softwa...

### How to Set Up a Run

Part of the in-the-lab instructional video series

### How to Carry Out a Band-Stab Procedure

Part of the in-the-lab instructional video series

### Best Practices for Sample Loading

Part of the in-the-lab instructional video series

## 📄 How to Connect the Base Fluid Line

Part of the in-the-lab instructional video series

## 📄 Steps to Remove Bubbles Introduced during Sam...

Part of the in-the-lab instructional video series

# AlphaFold

Seamless integration of AlphaFold into Nuclera's eProtein Discovery™ Cloud software, enabling a powerful, structure-guided approach to protein engineering.

Researchers can now go from sequence to purified, soluble protein in just 48 hours by combining AI-driven 3D structure prediction with automated screening. The intuitive interface allows users to visualize protein structures, analyze critical features like domain annotations, hydrophobicity, and residue-level confidence, and strategically design protein variants that balance function with manufacturability.

Key capabilities featured in this demonstration include:

- **Interactive 3D modeling** of predicted protein structures with zoom, rotate, and residue-level insights.
- **Dynamic visualization modes** to assess structural confidence, functional domains, and hydrophobic regions.
- **Integrated protein variant editor** to facilitate rational design and truncation/mutation decisions.
- **Bioinformatic validation tools** such as multiple sequence alignment, pLDDT, and PAE plots to guide expression optimization.

Through AlphaFold integration, protein design becomes a data-informed, high-efficiency workflow — empowering researchers to solve protein folding and solubility challenges with unprecedented speed and precision.

Loading...

# How to Set Up a Run

Part of the in-the-lab instructional video series

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# How to Carry Out a Band-Stab Procedure

Part of the in-the-lab instructional video series

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# Best Practices for Sample Loading

Part of the in-the-lab instructional video series

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# How to Connect the Base Fluid Line

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# Steps to Remove Bubbles Introduced during Sample Loading

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