eProtein Discovery™ System - Standalone

eProtein Discovery system

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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 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!

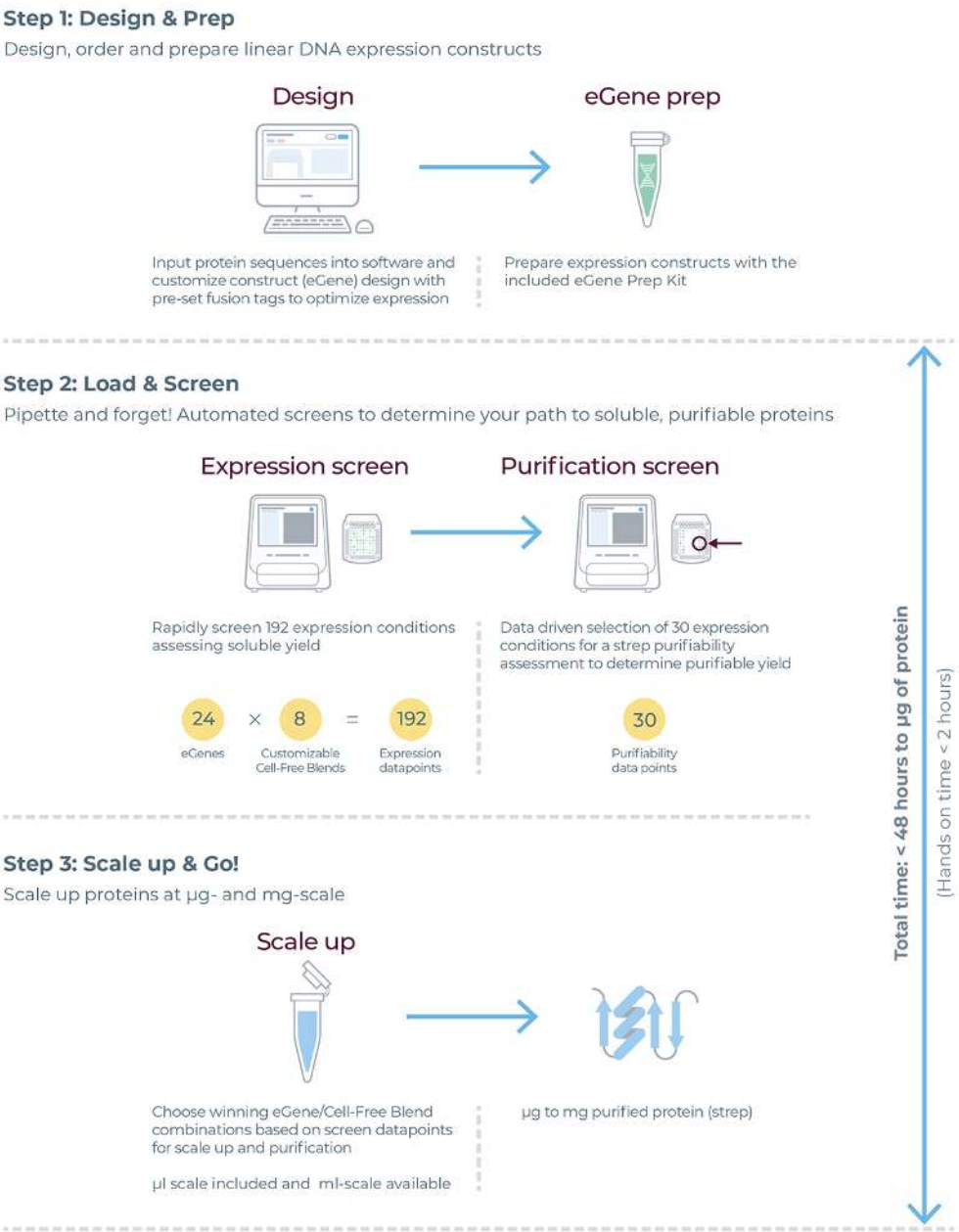
Software: eProtein Discovery™ software simplifies a complex multivariate experimental design. The software sets up and simultaneously tracks 192 different combinations of DNA sequences, flank pairs and expression reagent reactions performed on eProtein Discovery™ system. AI performs highly rigorous QA 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. • 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 • 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. 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 • If 1 TMD present remove domain * If >1 TMD present discard Candidate sequence • If N-term TMD truncate after TMD (e.g. aa directly after TMD) • 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) + List of Child Candidates (step 4)		• Consider N-terminal truncation to leave the domain of interest. Users may add up to 10 aa upstream. • Consider C-terminal truncation to leave the domain of interest. Users may add up to 10 aa downstream. • 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. • Consider truncating to remove disordered regions around functional domain of interest • 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) • Consider your cartridge format (e.g. a FlexiVariant™ or Solubility tag screen • Consider your cartridge budget (e.g. do you plan on running one or more cartridges within this campaign) • 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.

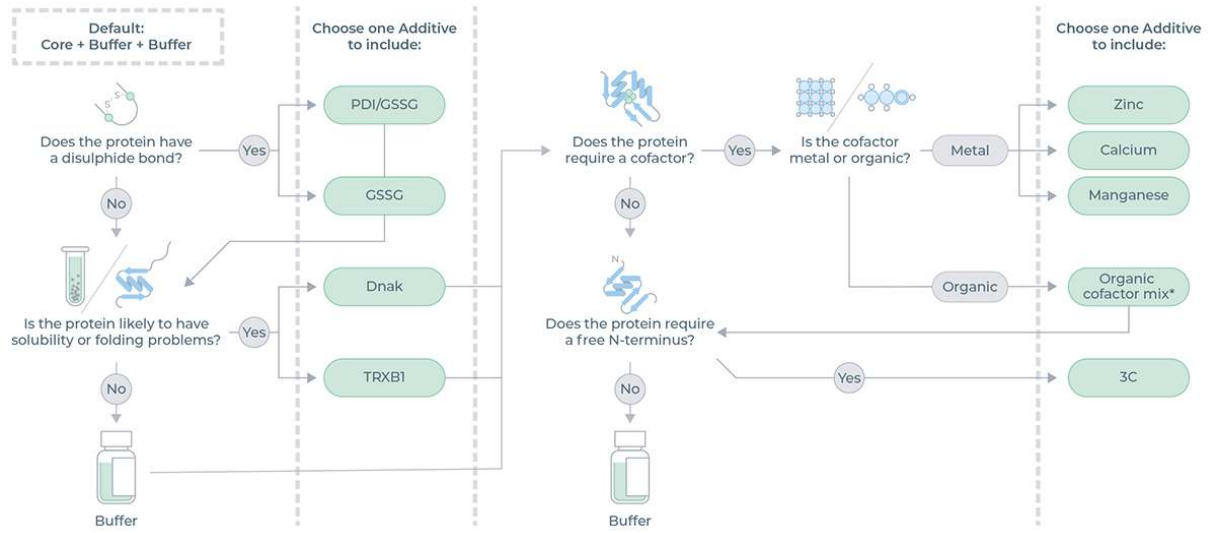


Figure 1: Decision tree which illustrates the Additive options based on protein requirements.

*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.

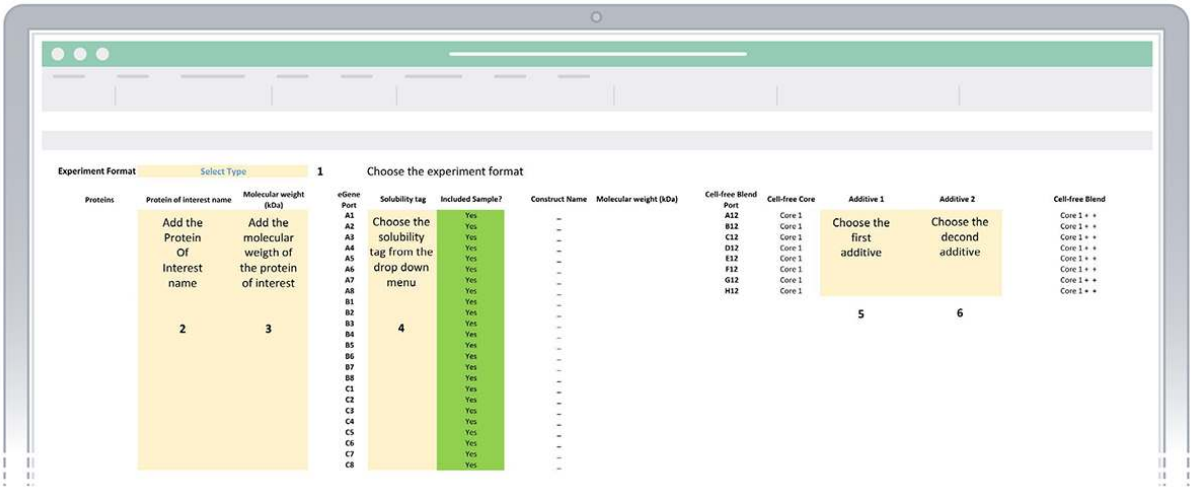
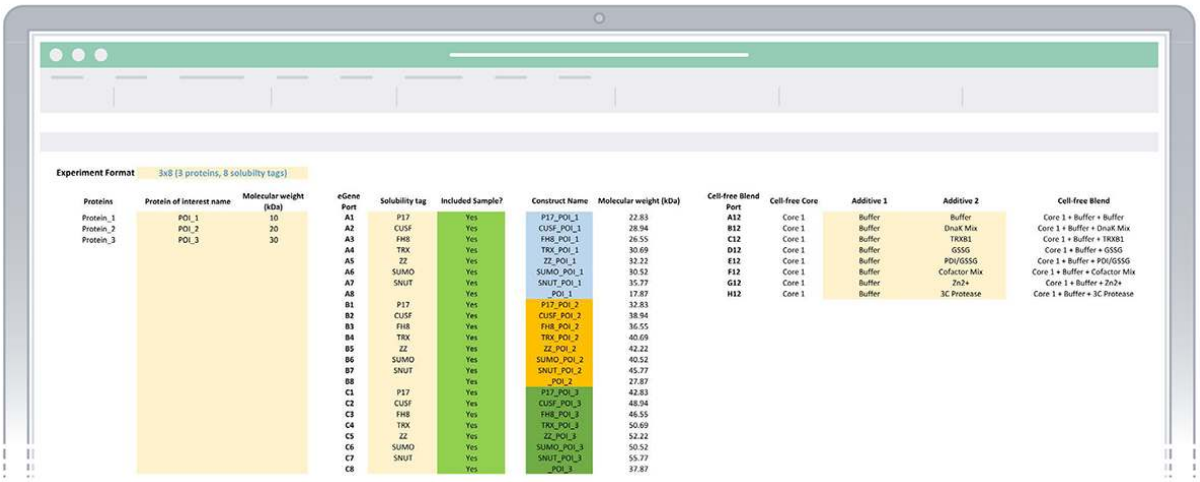


Figure 2: Sheet “1. Enter experiment details” of the eProtein Discovery Standalone template.

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.

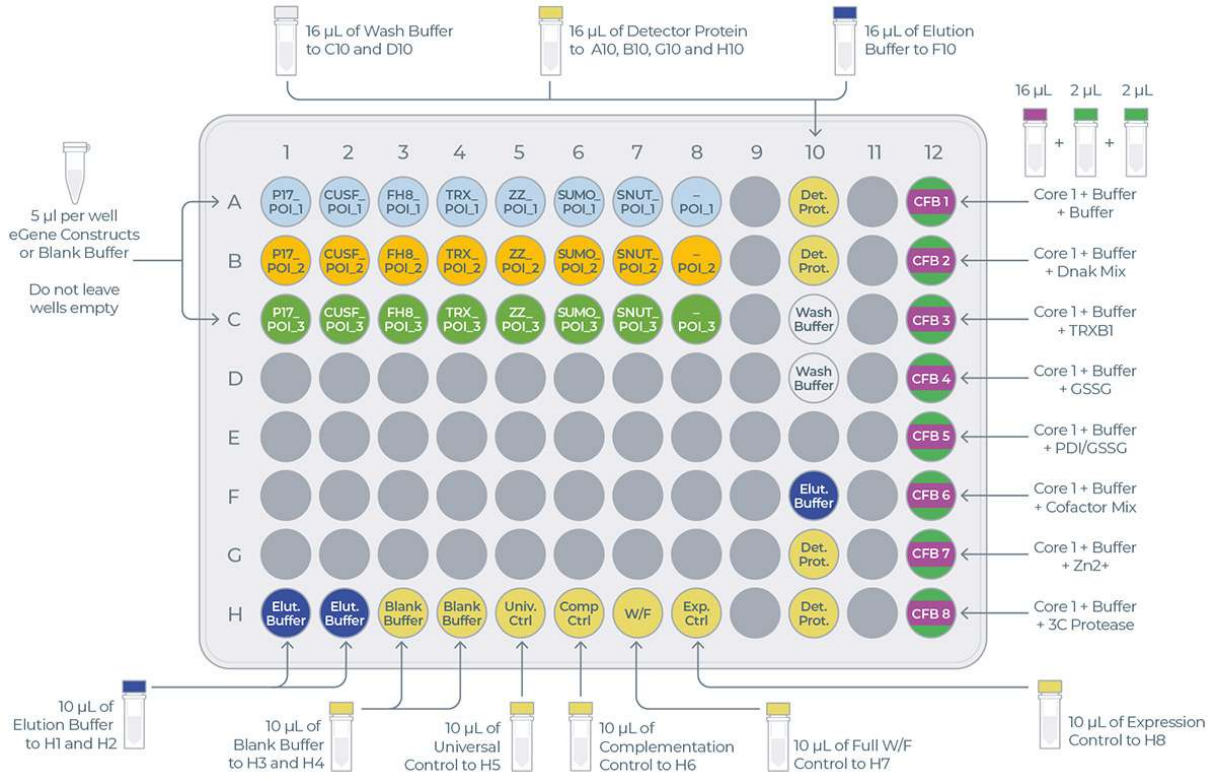


Figure 4: Example of transfer plate design

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).

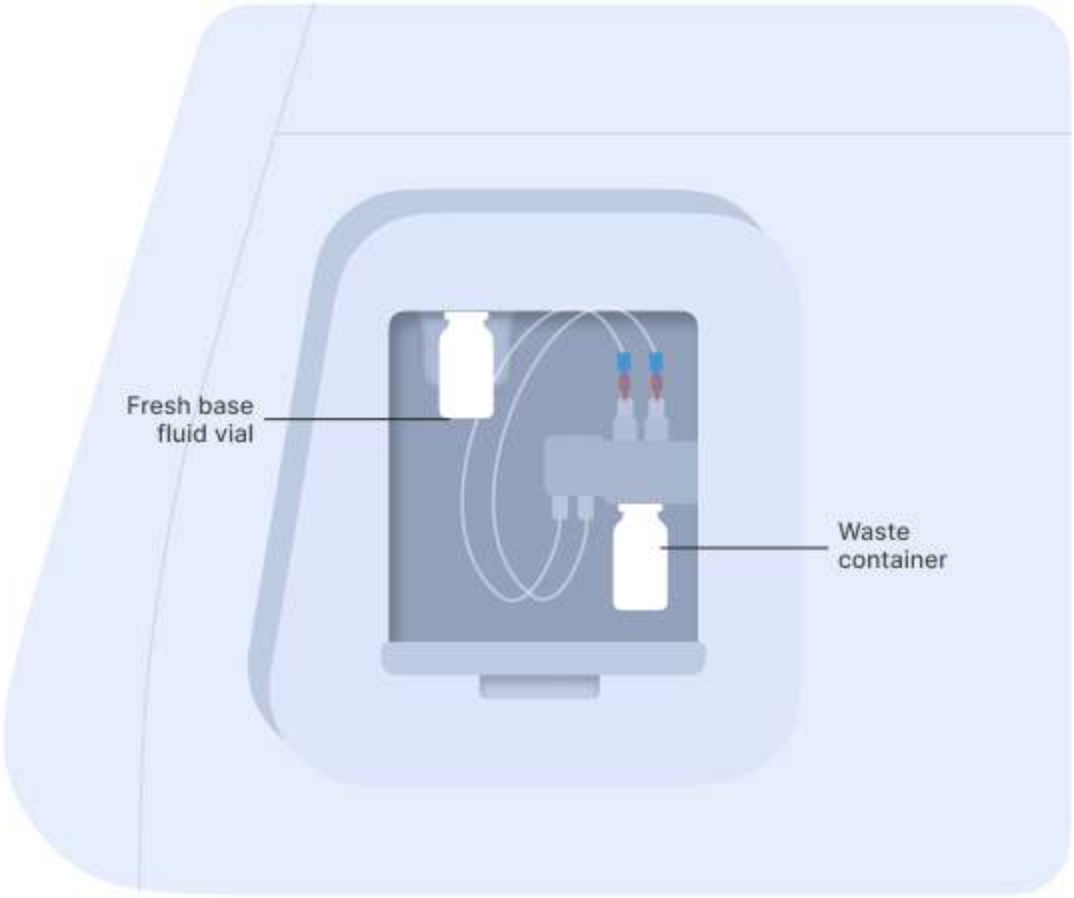


Figure 5: Vial of base fluid and the waste container connected to the pump as shown on the screen

[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, ake 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.

NOTE

If a eGene construct is missing it must be substituted with 5 µL eGene Elution Buffer supplied in the eGene Prep kit.

IMPORTANT NOTE

Do not substitute a missing eGene construct with water, use Additive Buffer instead.

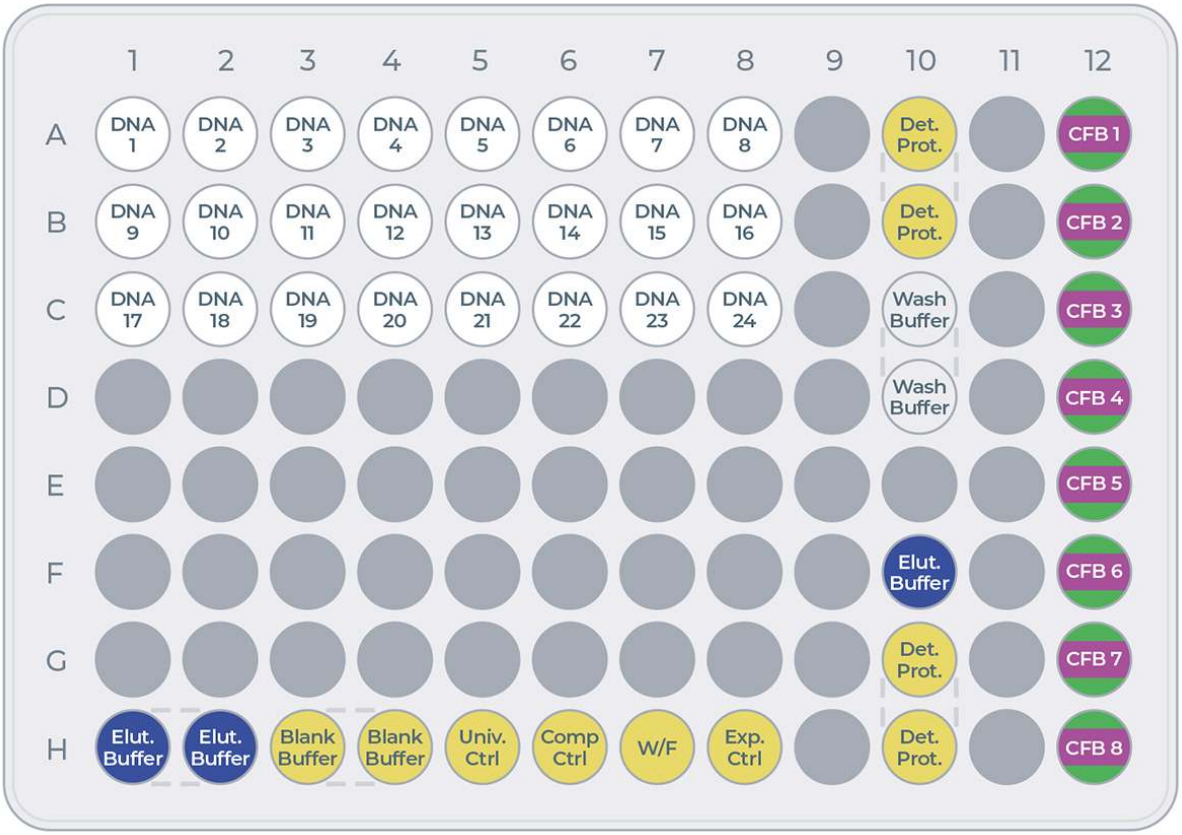


Figure 7: Transfer plate layout

Reagent	Volume (µL)
eGene construct	5
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)

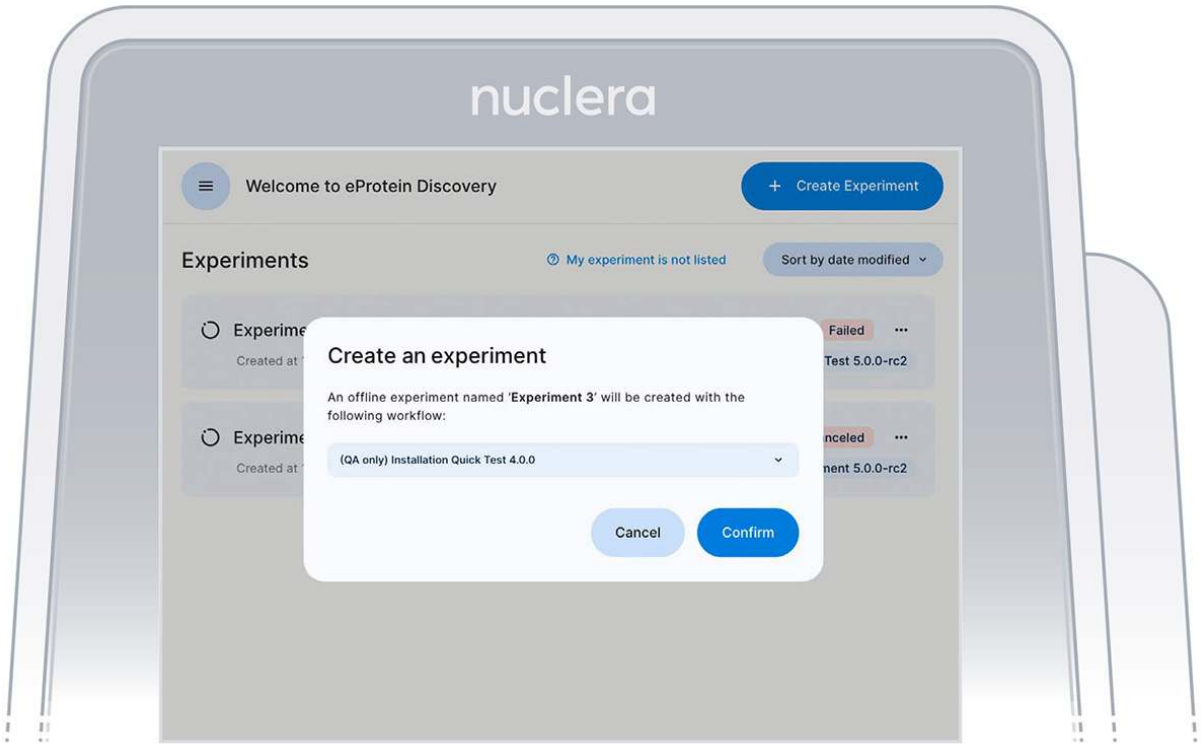


Figure 8: Experimental design screen

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.

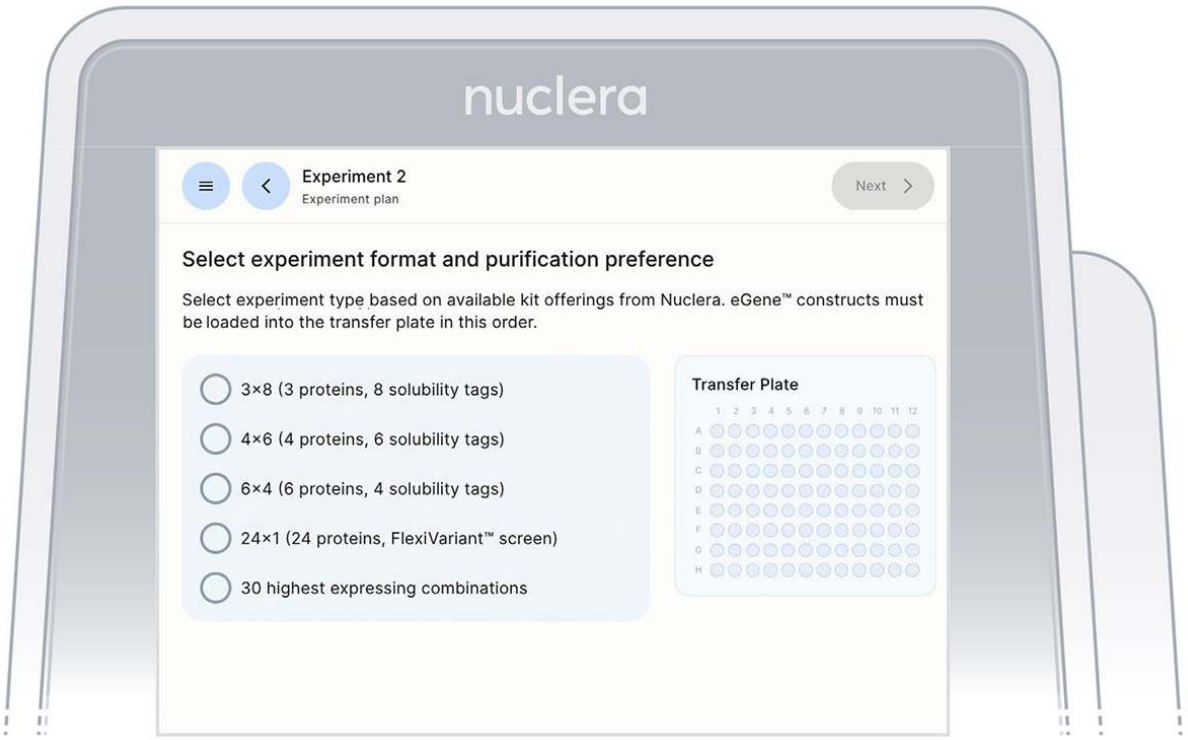


Figure 9: Instrument screen to select the experiment format

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

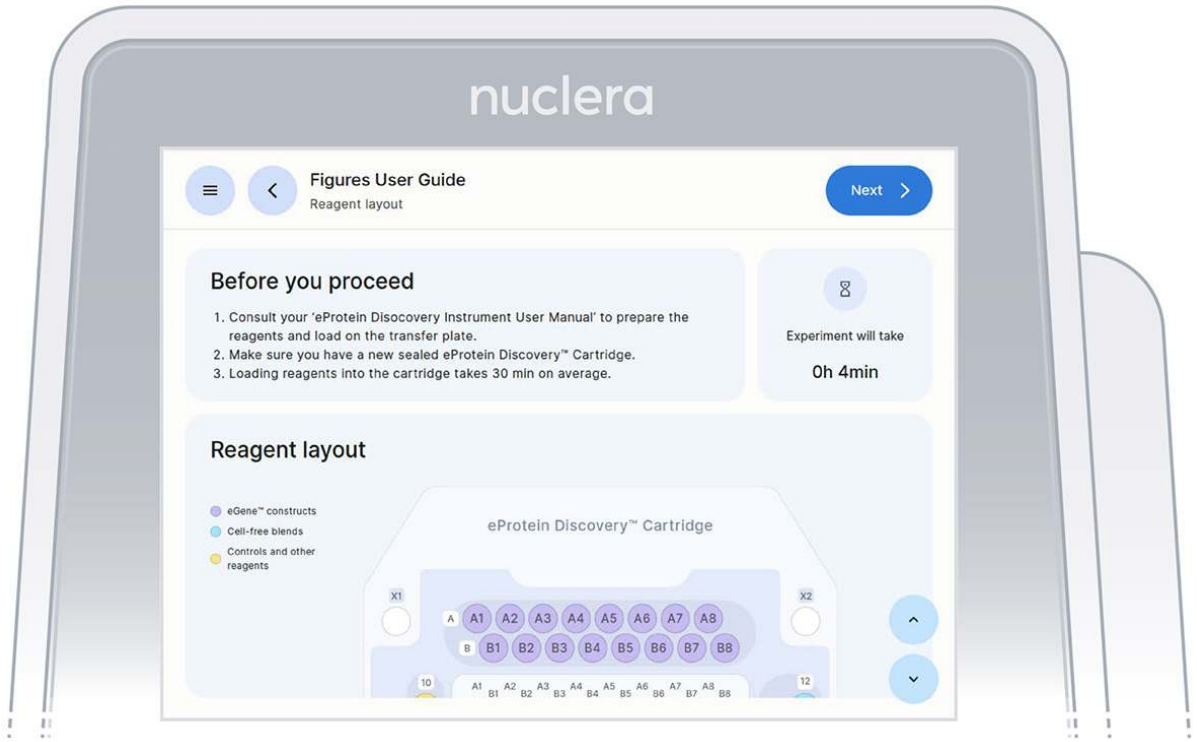


Figure 10: Summary of the selected experiment

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.

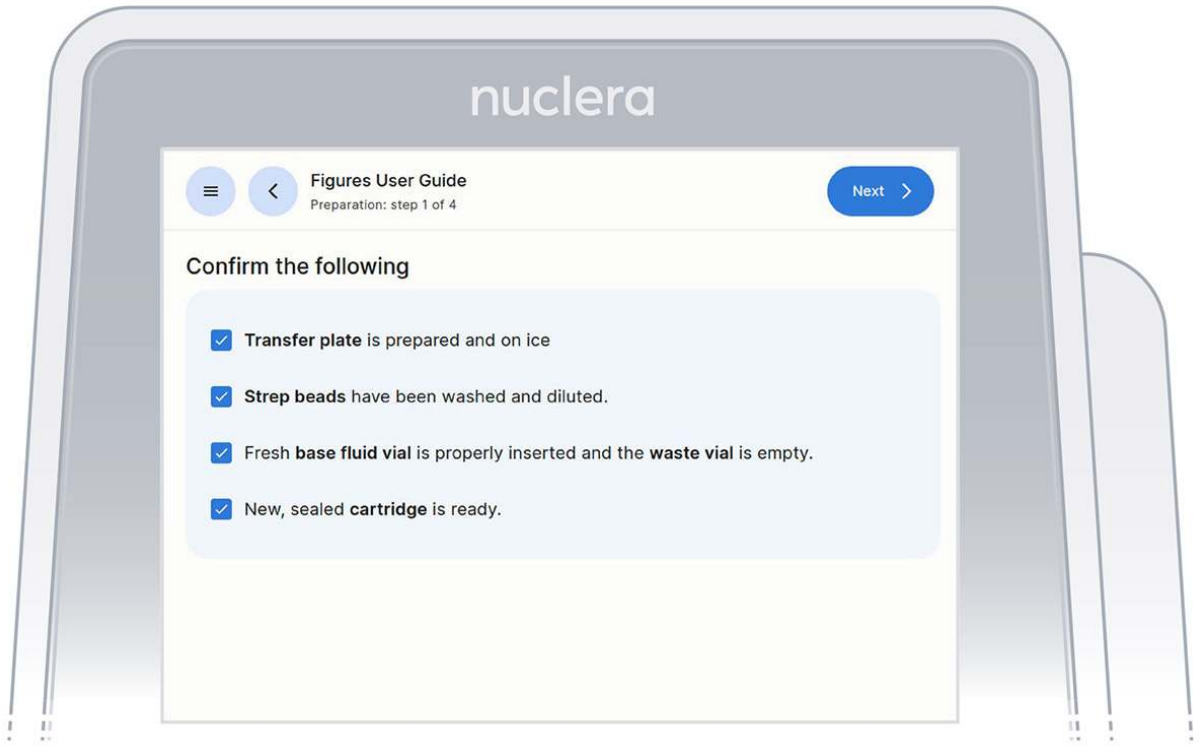


Figure 11: Checklist screen before the experiment starts

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).

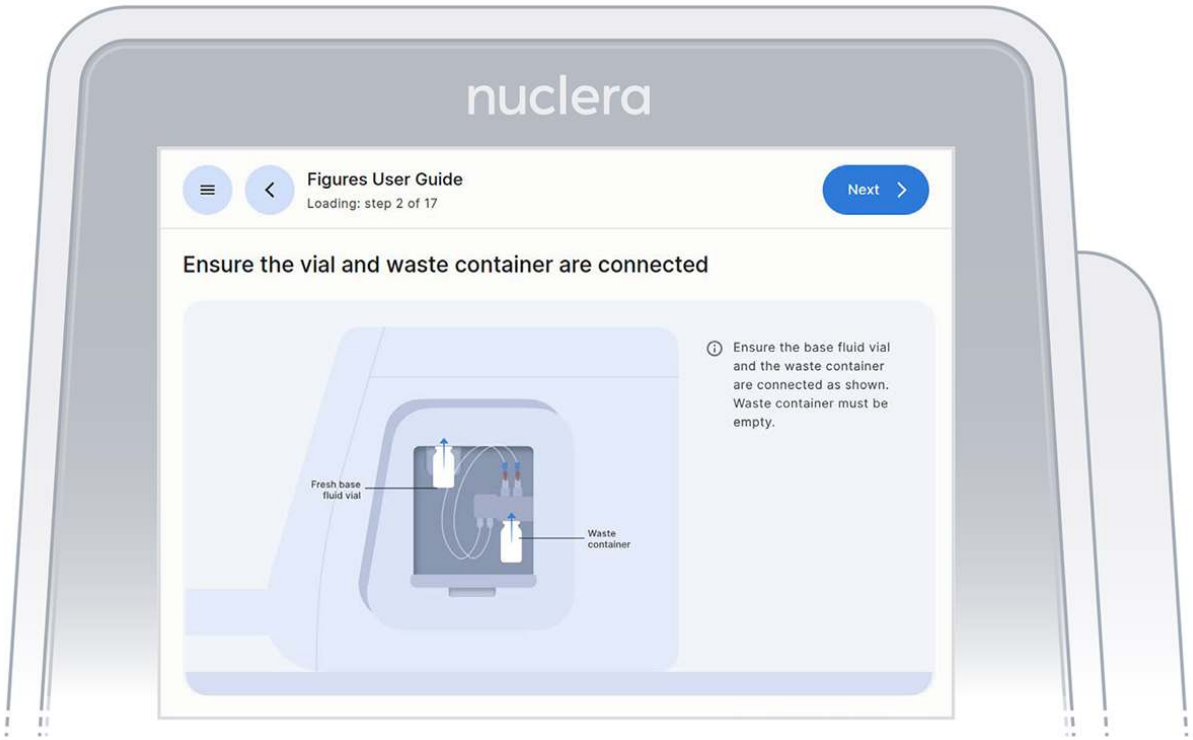


Figure 14: Vial of base fluid and the waste container connected to the pump as shown on the screen

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).

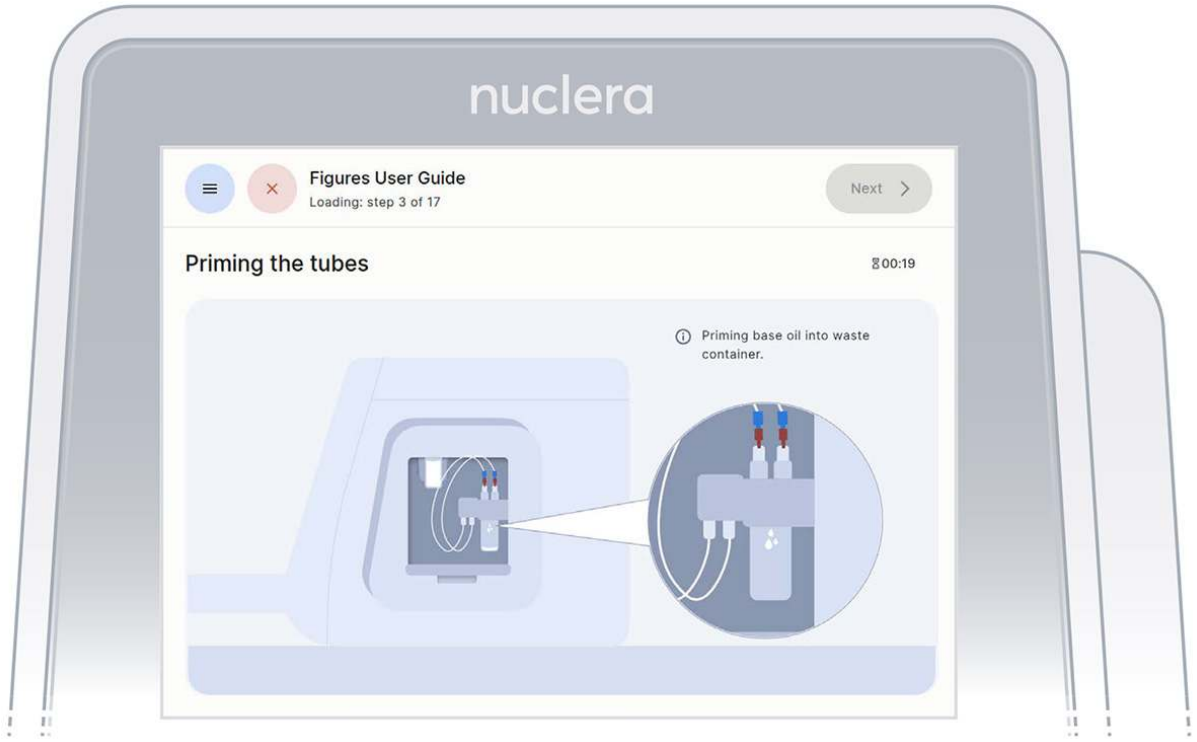


Figure 15: Priming the pump tubings with base fluid

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.

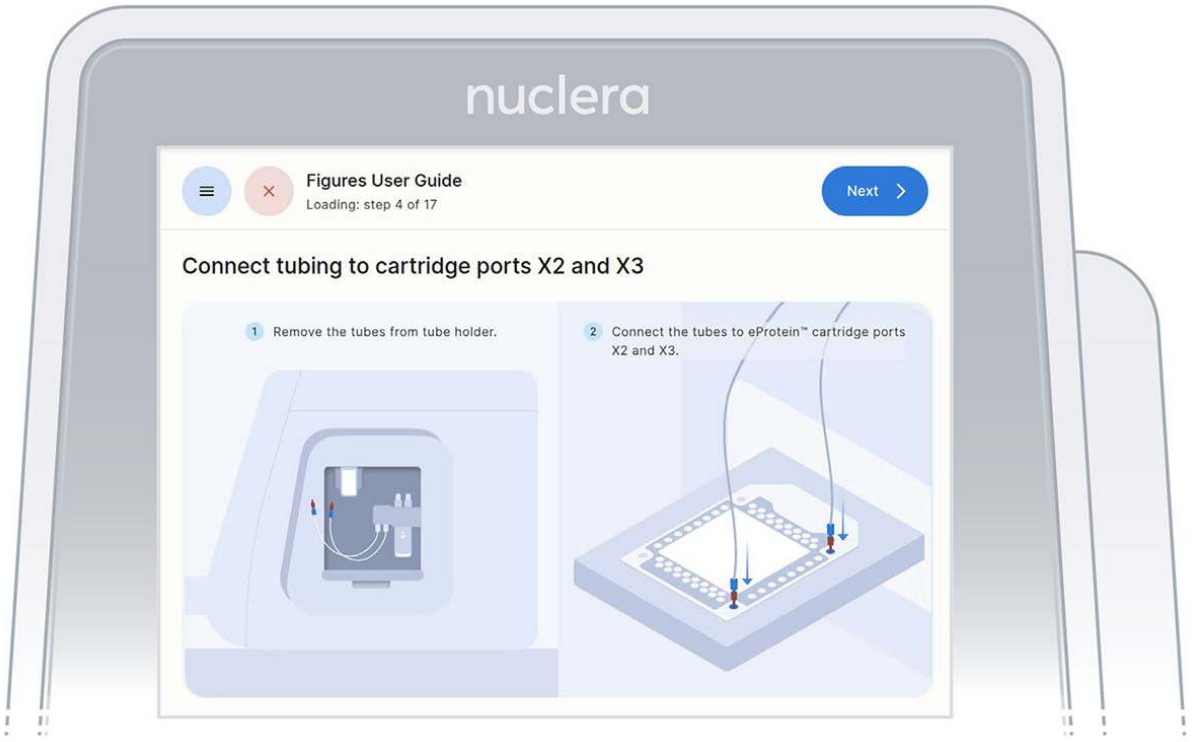


Figure 16: Connection of the pump tubes to the cartridge

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).

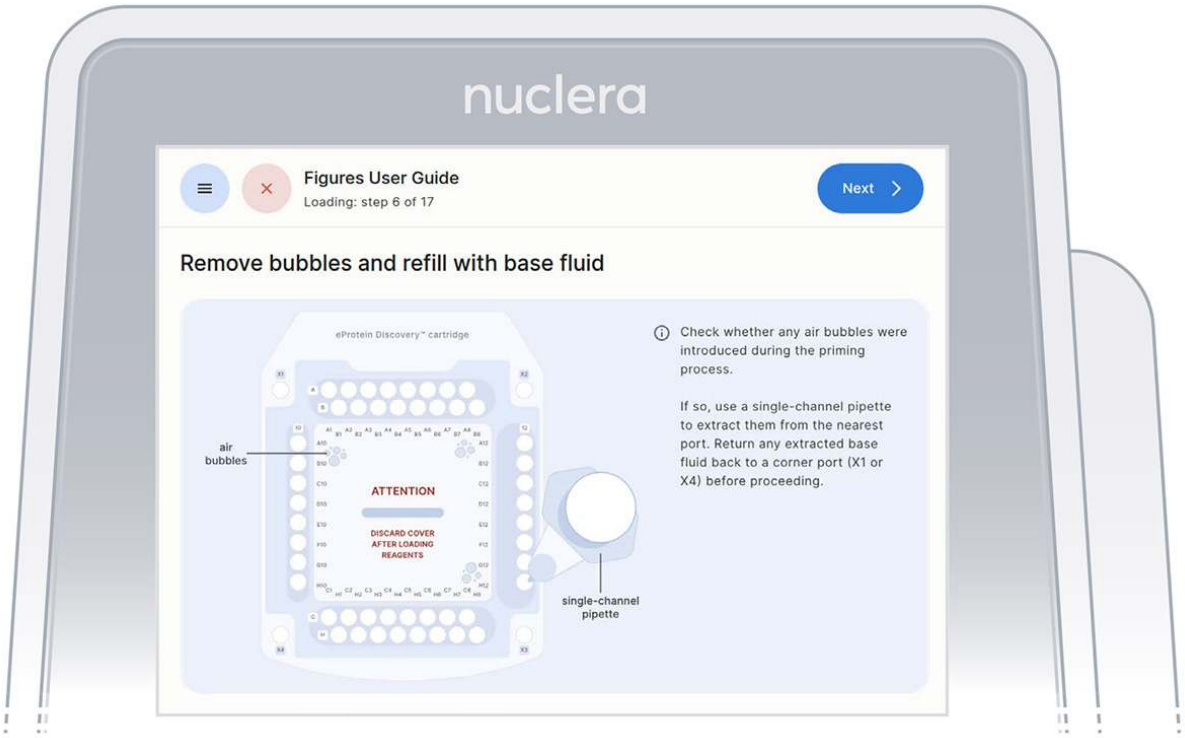


Figure 17: Procedure to remove potential air bubbles from the cartridge

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).

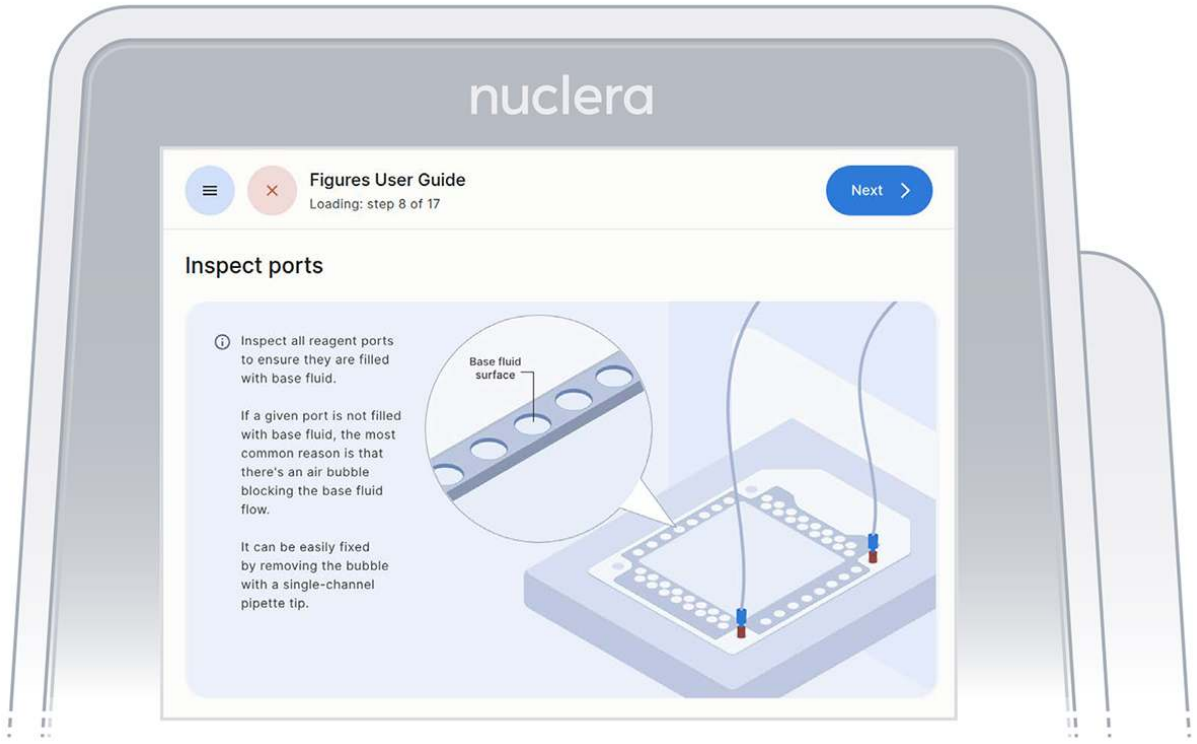


Figure 18: Procedure to remove potential air bubbles from the cartridge

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).

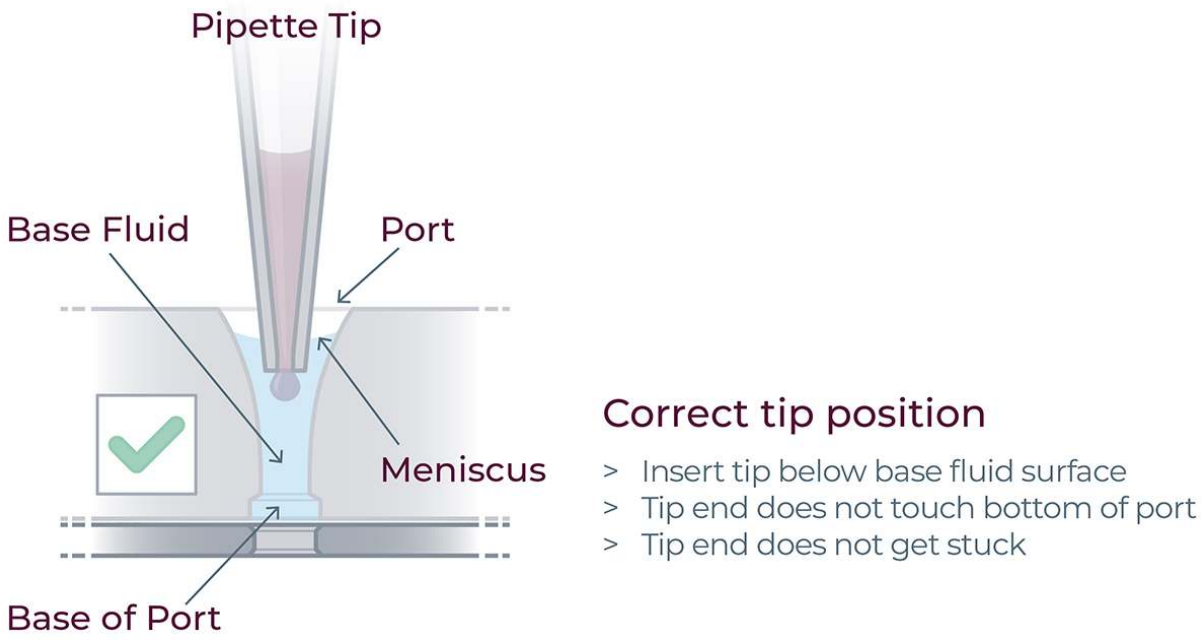


Figure 19: For correct reagent loading the pipette tip is immersed in the base fluid and not touching the bottom of the port

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

▹ 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.

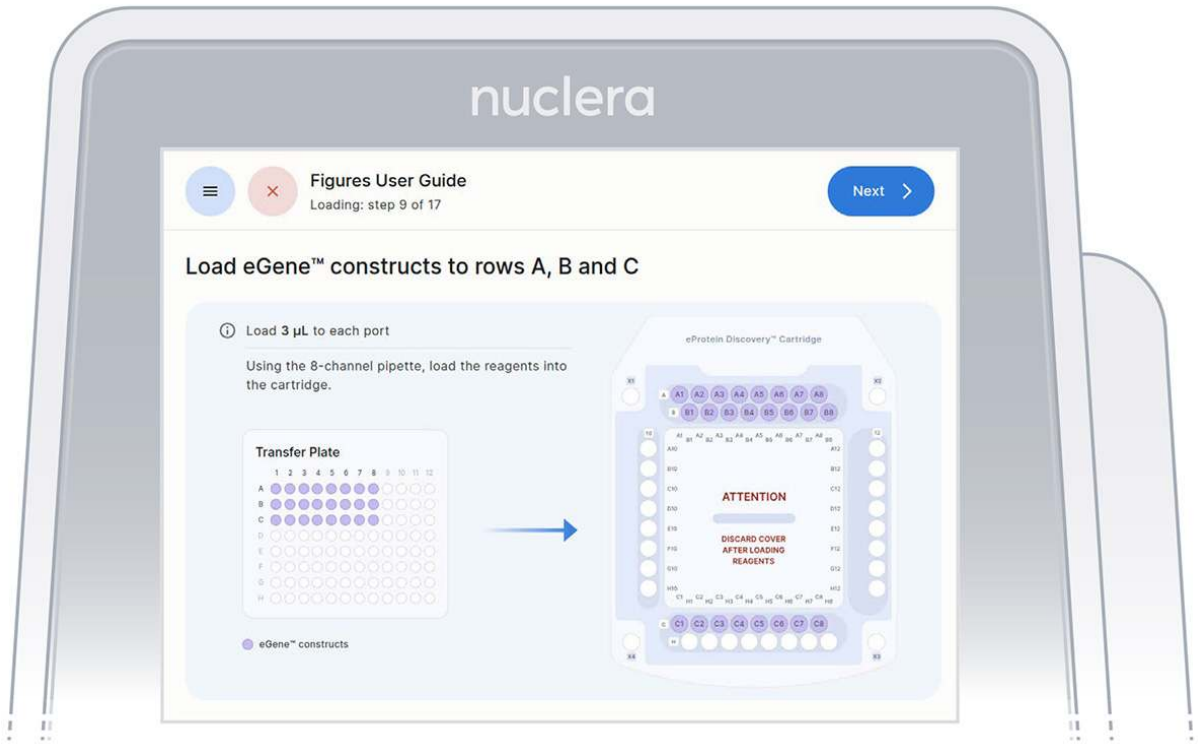


Figure 20: Loading of the eGene constructs onto row A, B and C of the cartridge

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.

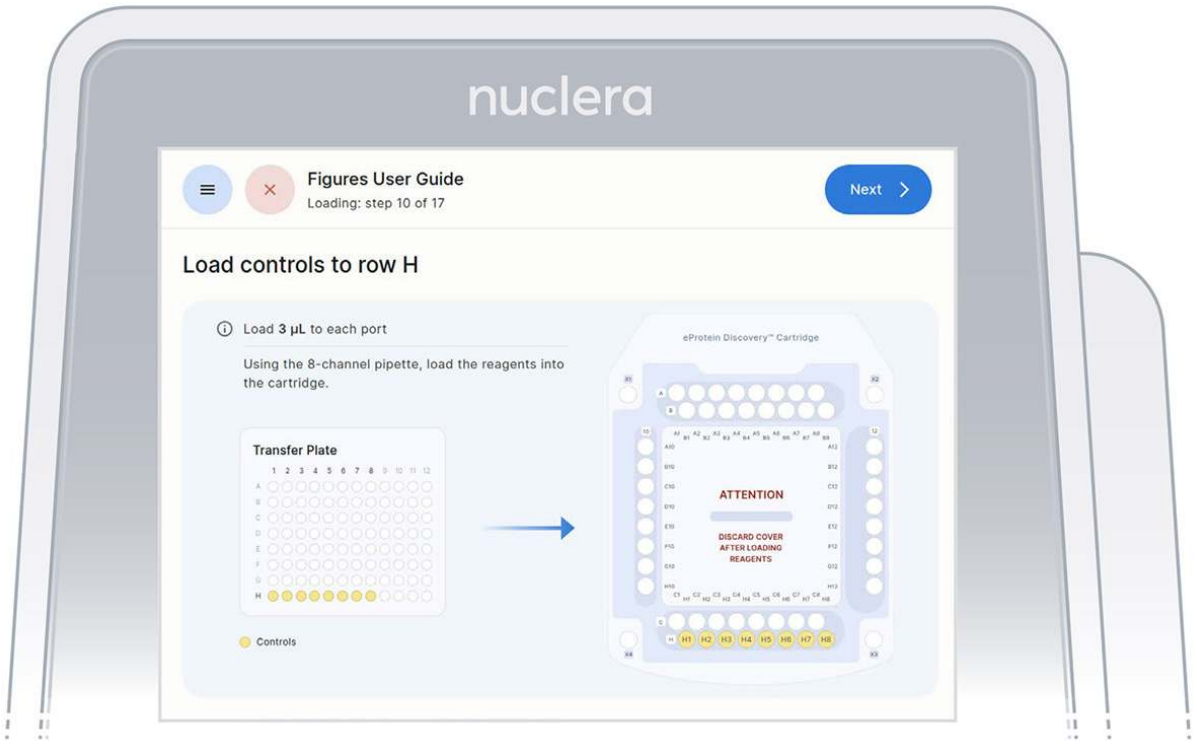


Figure 21: Loading of the reagents onto row H of the cartridge

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).

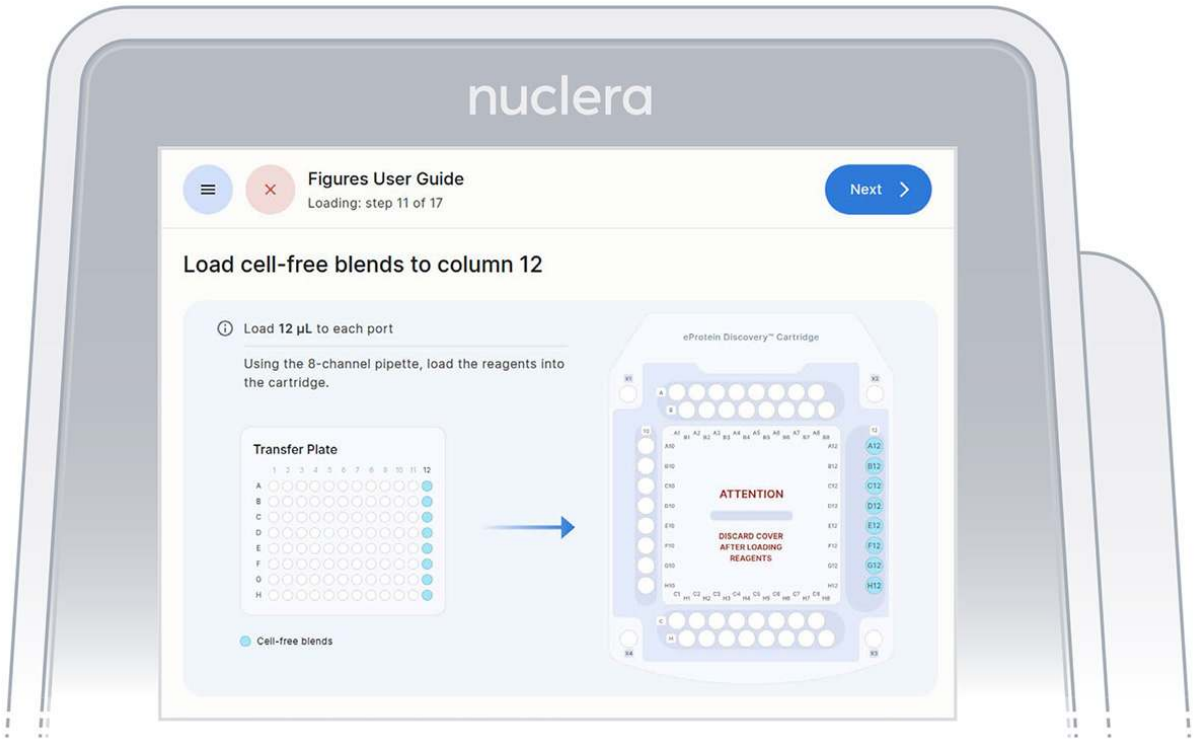


Figure 22: Loading of the Cell-free Blends onto column 12 of the cartridge

▹ 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.

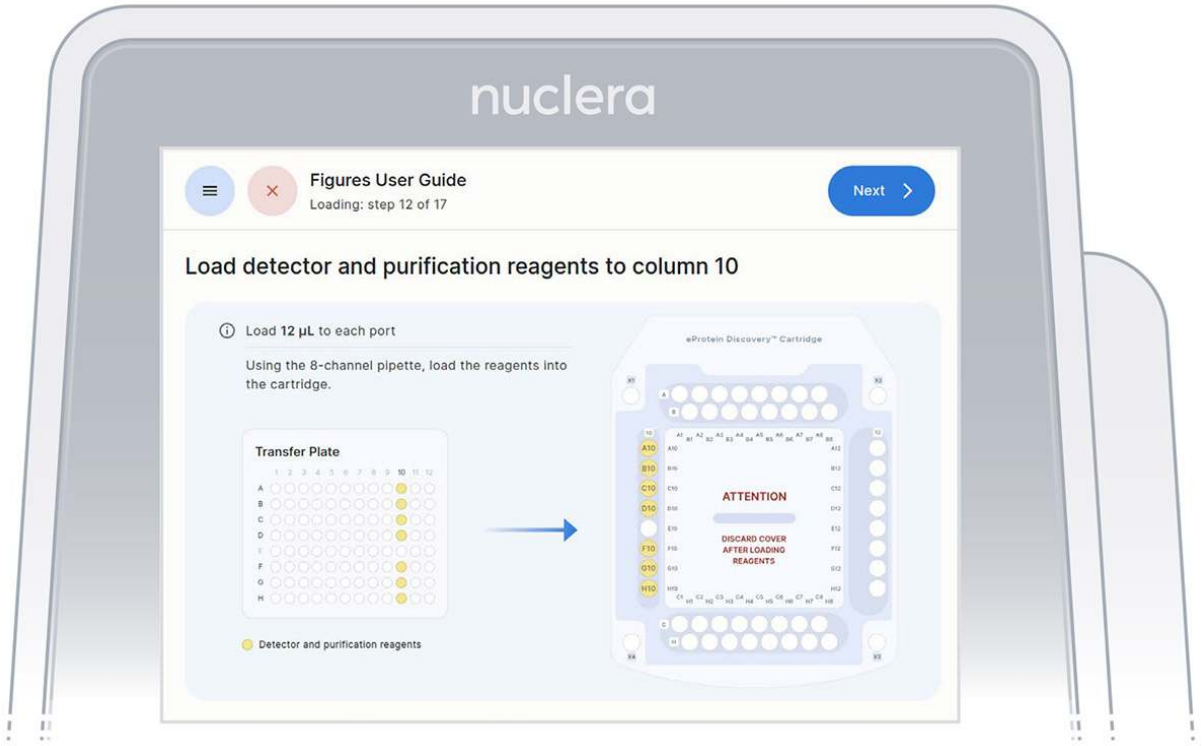


Figure 23: Loading of the reagents onto column 10 of the cartridge

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.

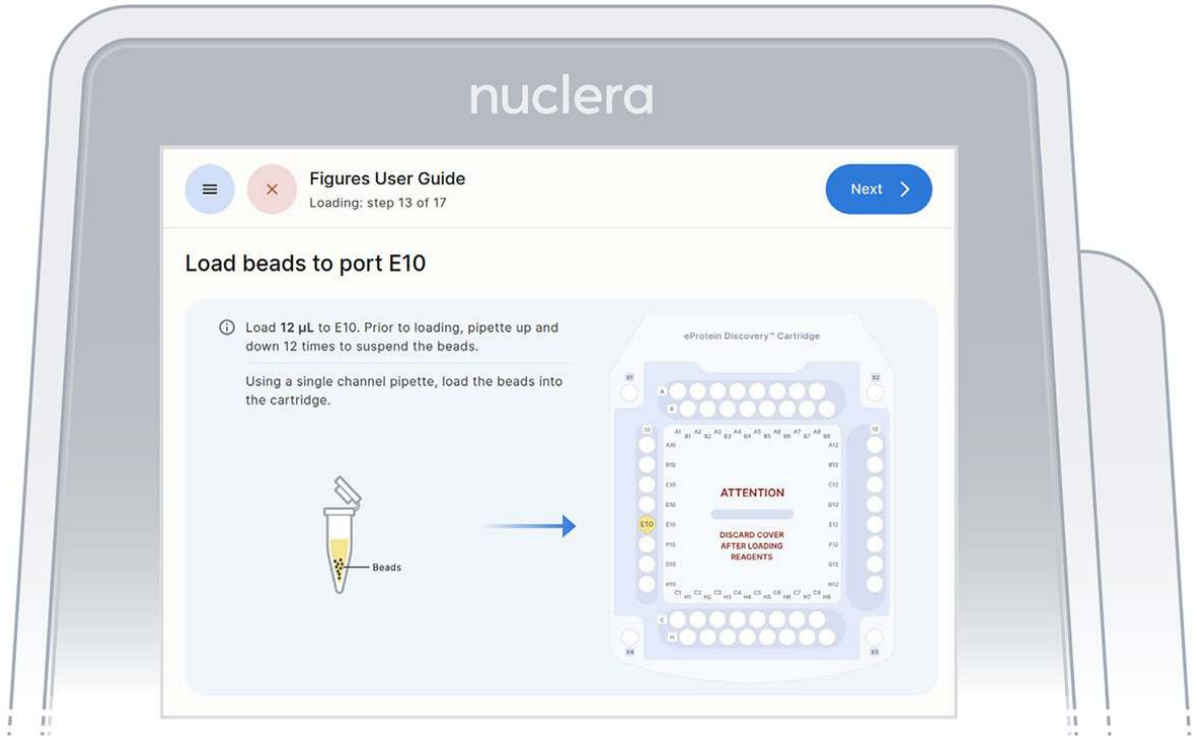


Figure 24: Loading of the Strep beads to port E10 of the cartridge

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

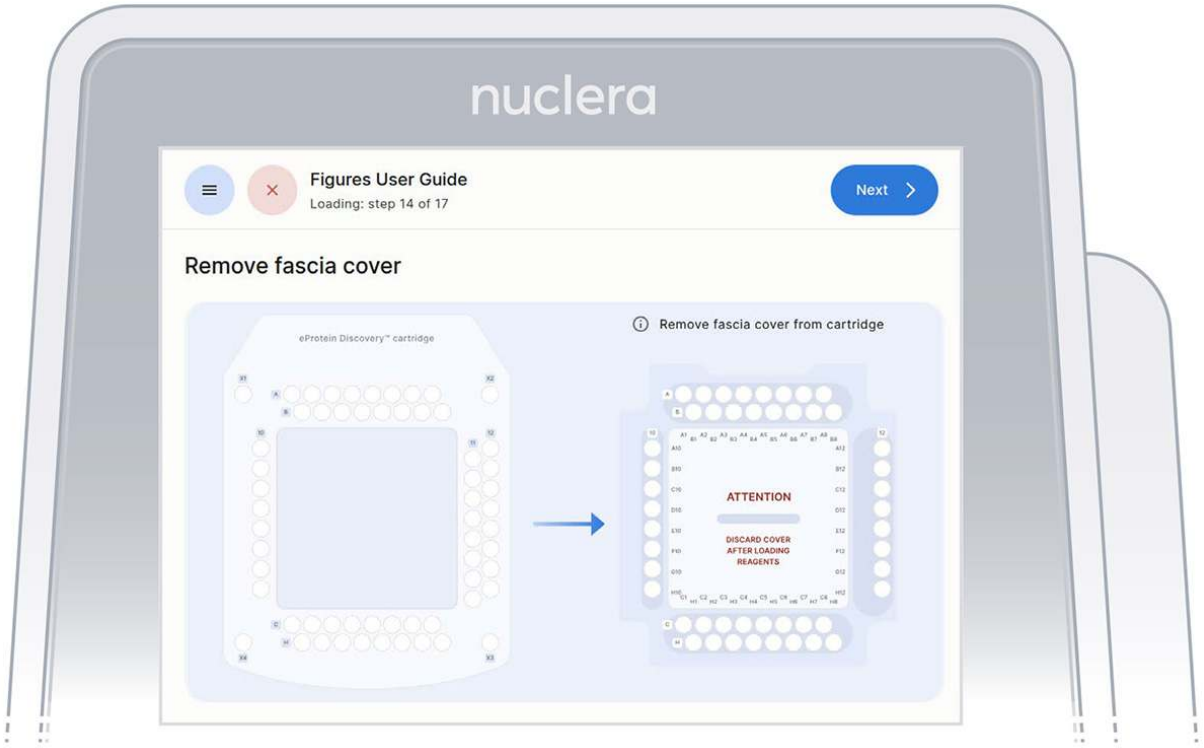


Figure 25: Remove the cover from the cartridge

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).

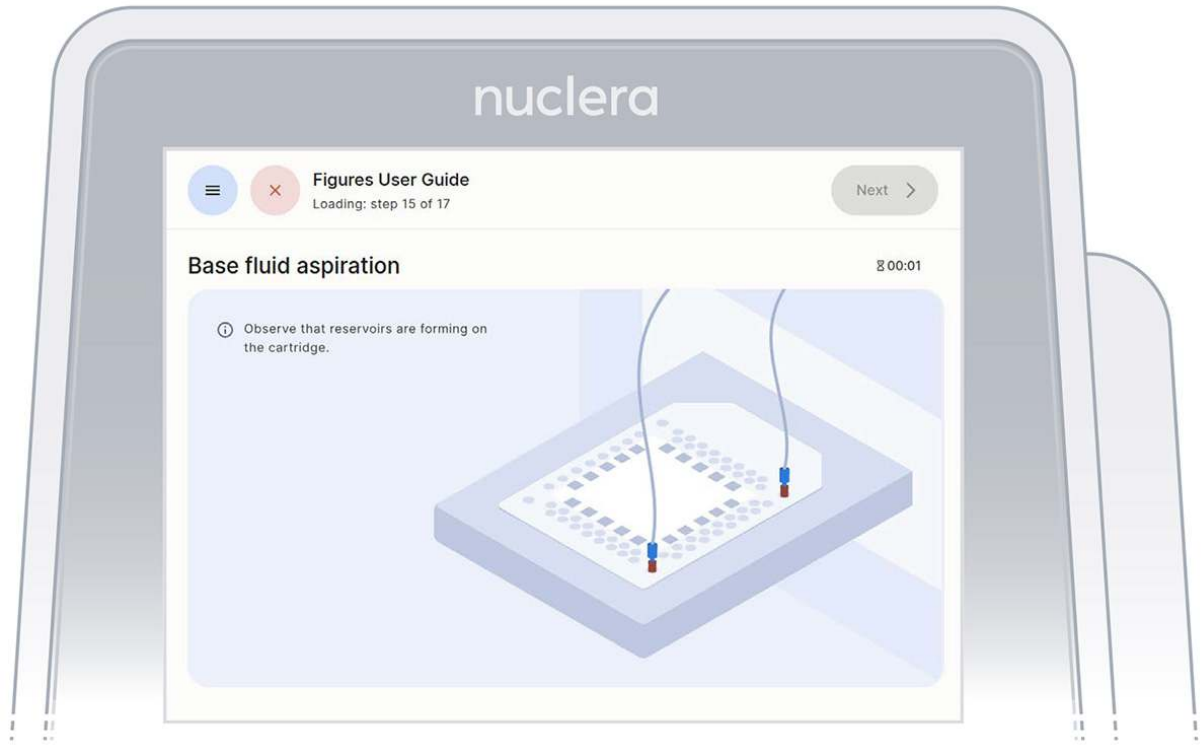


Figure 26: Base fluid aspiration

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.

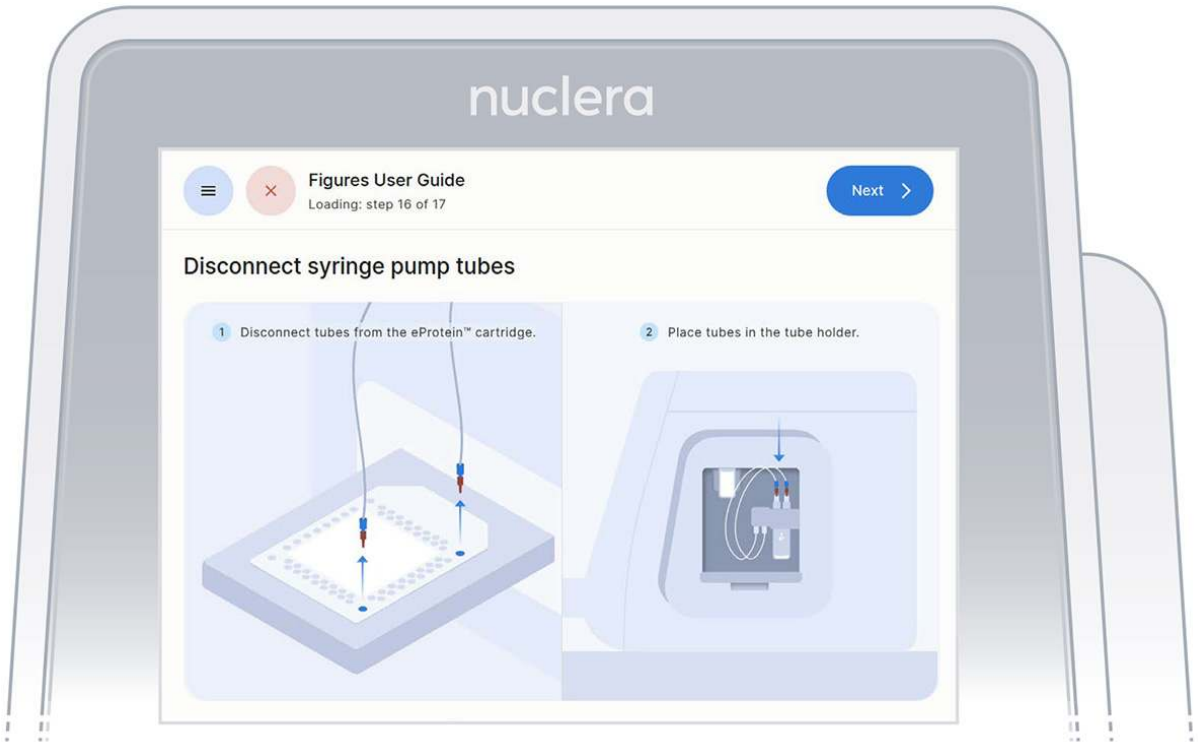


Figure 27: Disconnect the tubes and place them on the tube holder

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

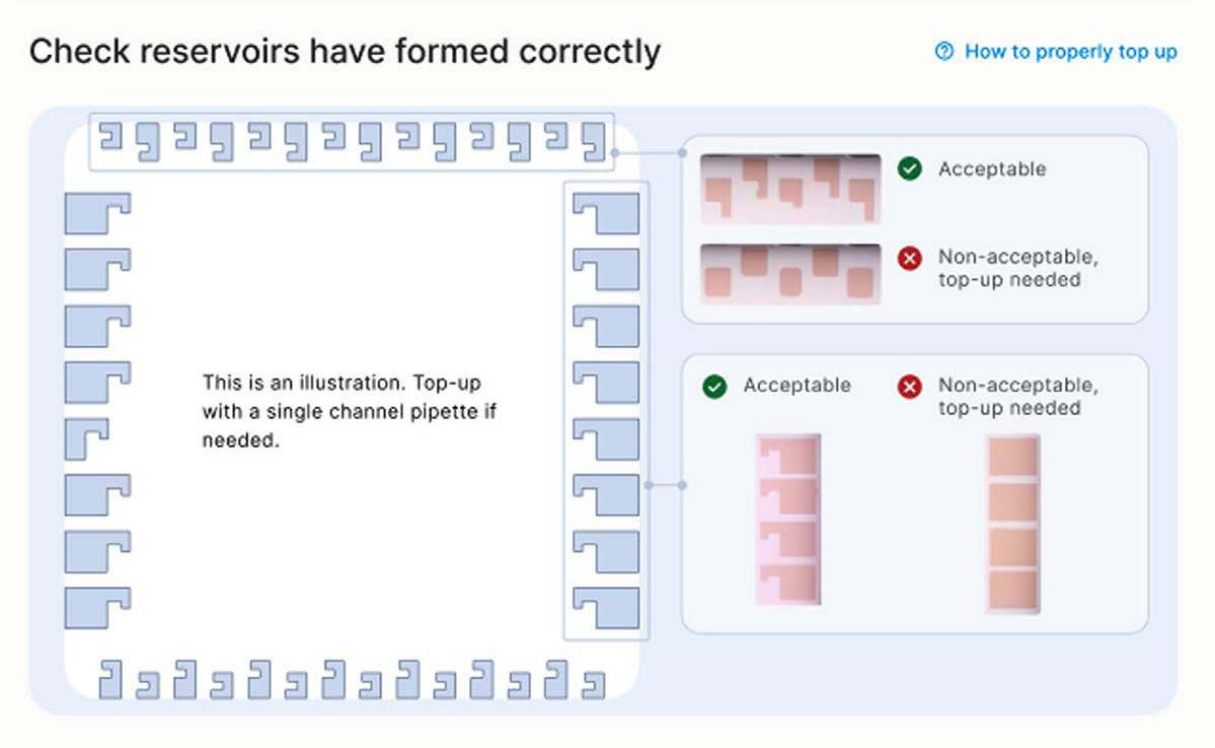


Figure 28: Check the reservoirs have formed correctly on the cartridge

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

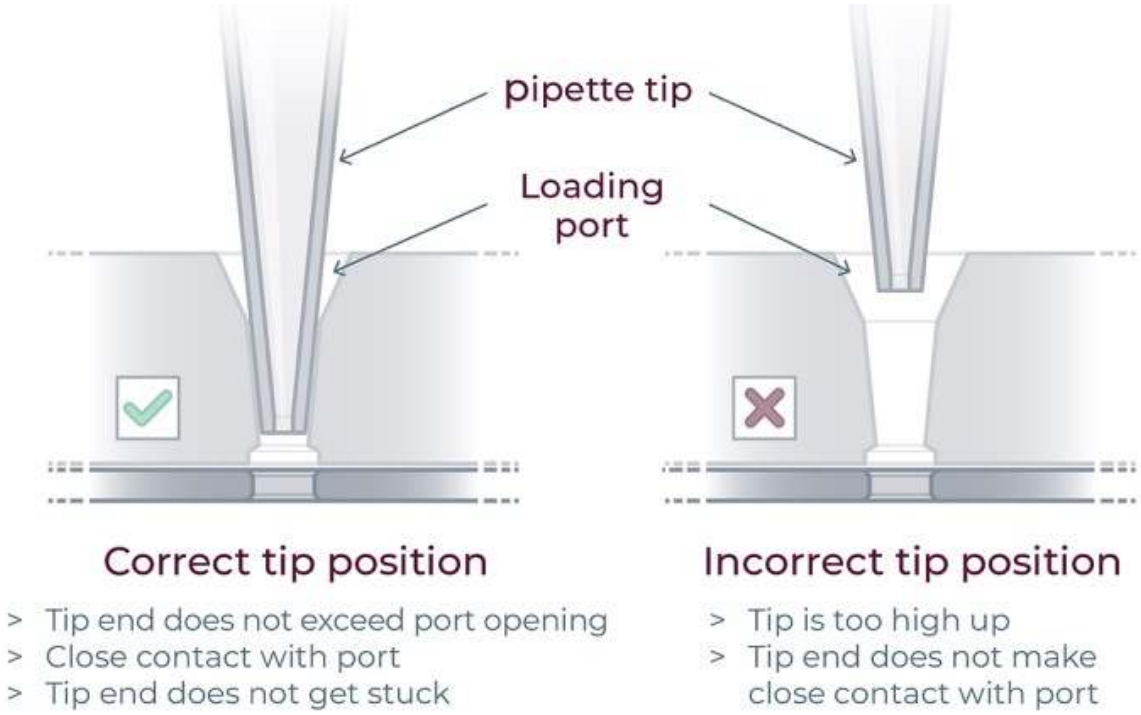


Figure 29: For manual correction of the reagent loading to correctly form the reservoirs on cartridge

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).

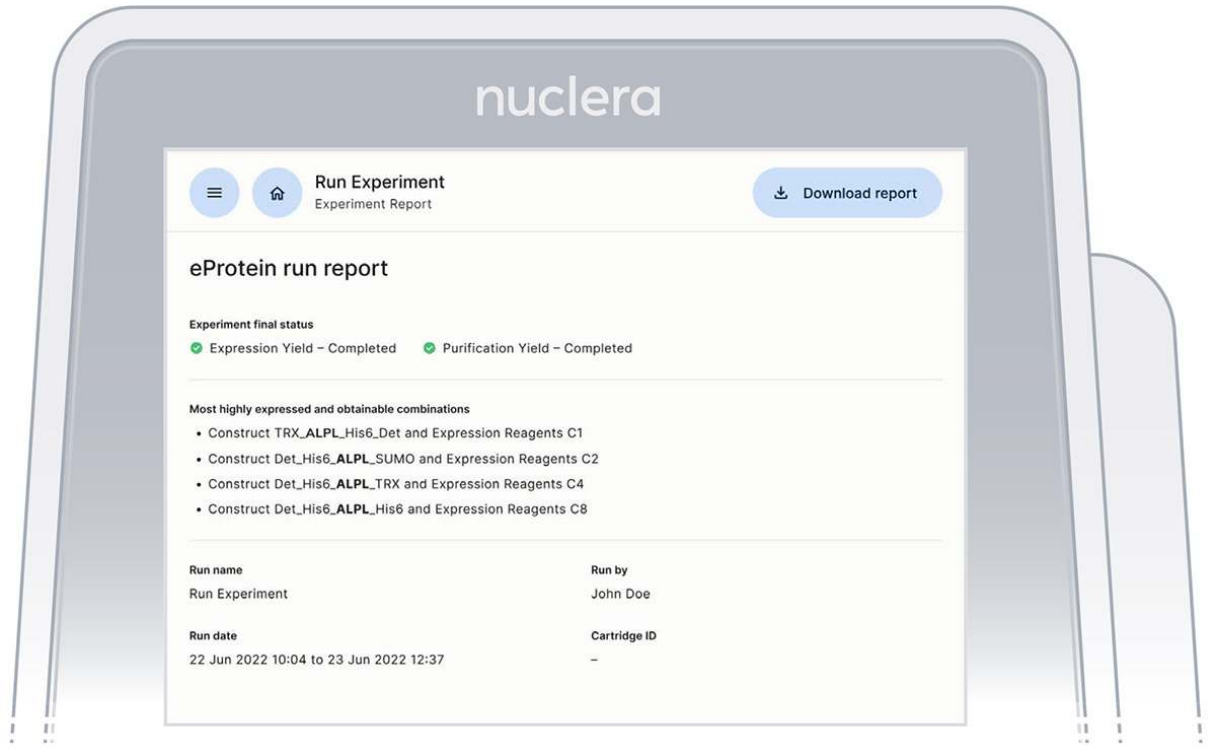


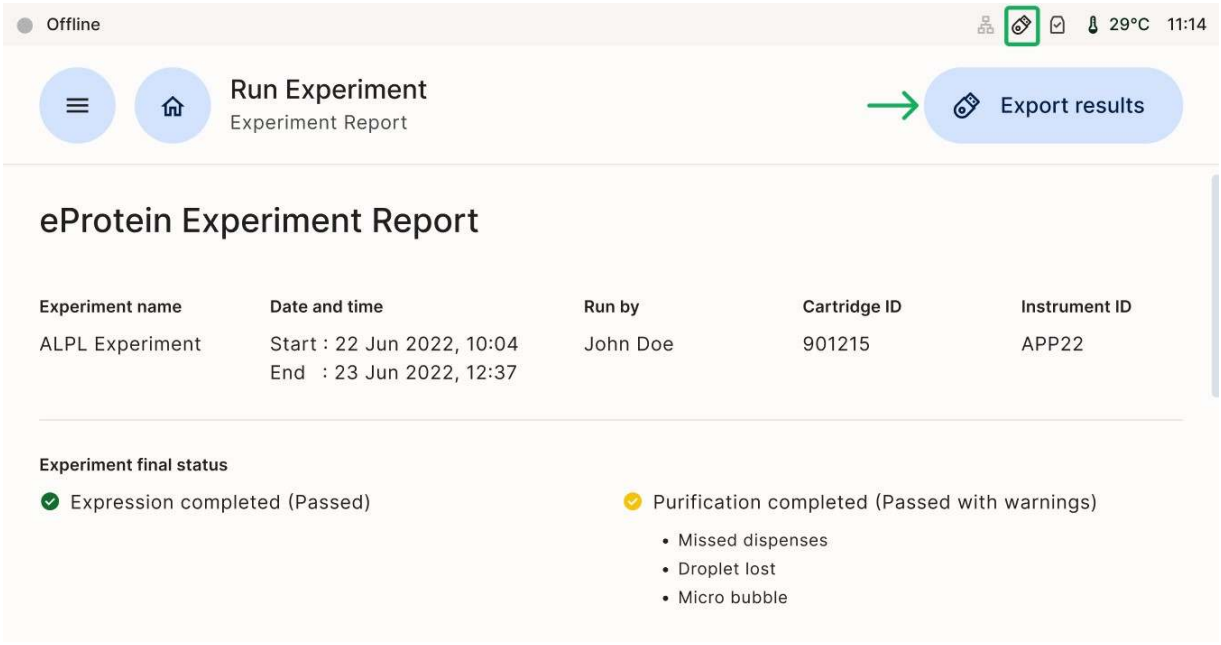
Figure 30: Result screen from the Instrument Software

eProtein Discovery report

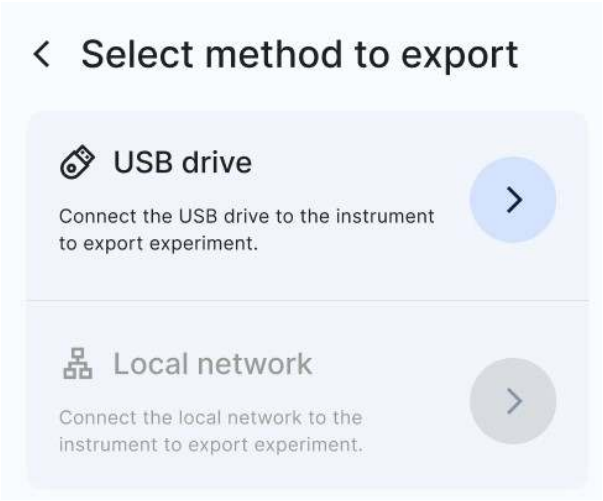
At the end of the experiment a report containing all the information about the experimental setup and the results can be downloaded from the instrument. Data can be exported using a USB, LAN or a Local Laptop. The upload takes about 15 minutes and during this time the [Download Report] button at the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

USB

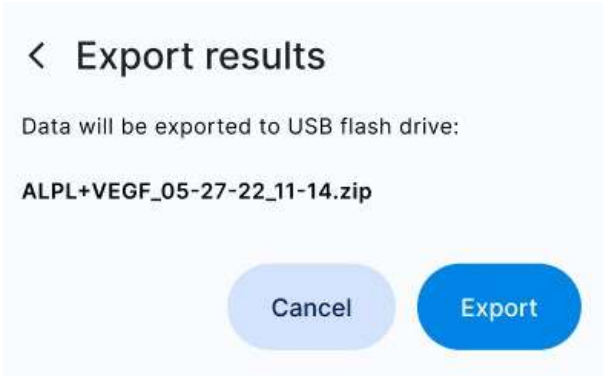
To export data to a USB flash drive it required to have a company-approved USB flash drive for data transfer. Encrypted flash drives are not currently supported. Insert the flash drive on the right hand side of the instrument in the USB port. This will highlight the flashdrive icon on the screen. Click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

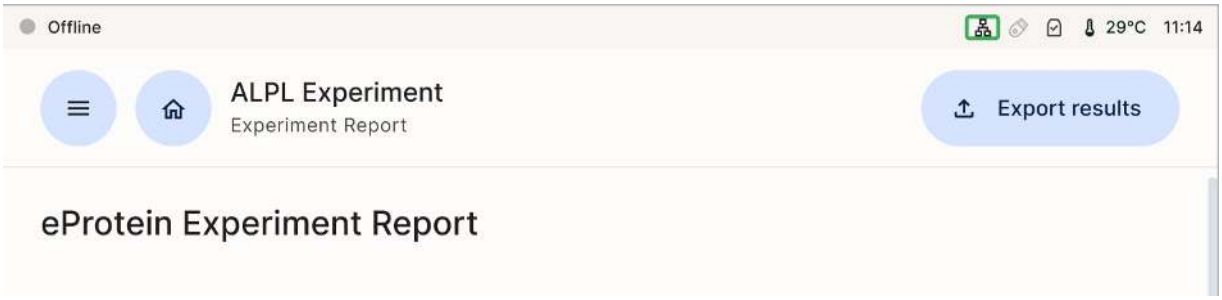


(b)

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

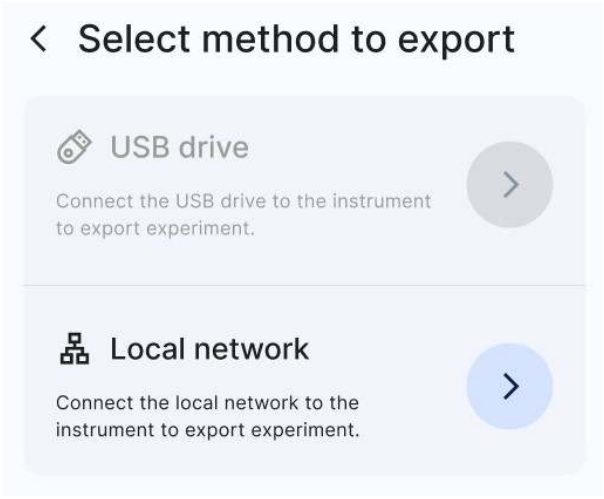
LAN and Local Laptop

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.

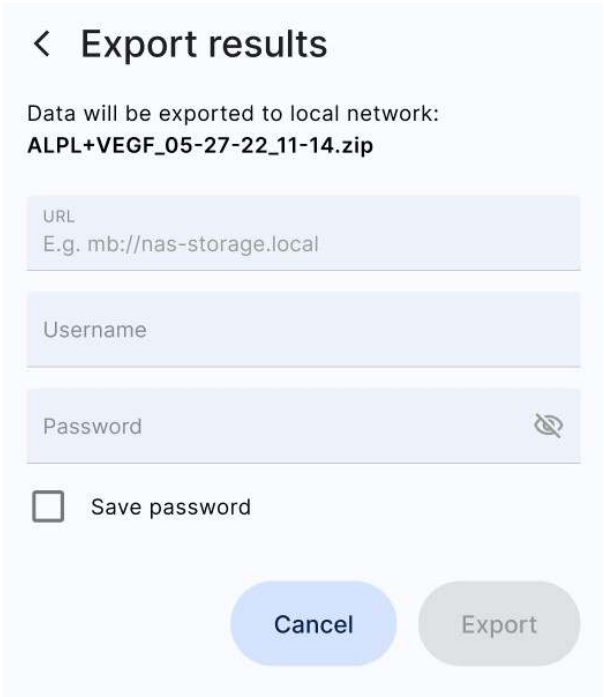


Open a completed experiment and tap the “Export results” button.
Tap (a) "Local network" and enter the following (b):
URL: smb://192.168.1.100/eProteinReports
Username: the name of the Windows user that has access to the shared directory, if this is a domain user then the username should be of the form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

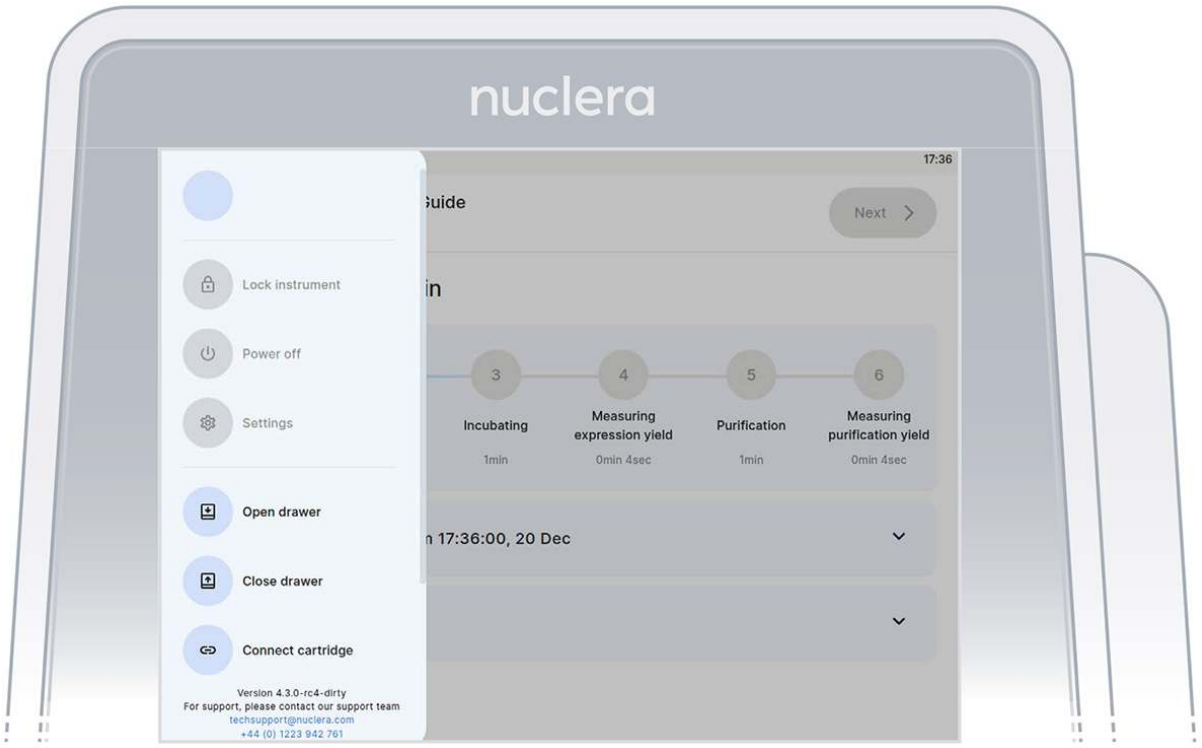


Figure 32: Remove the cartridge from the instrument

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

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 • Scale-up Additives (list in Table 2) • 5 nM eGene™ construct (DNA)	30 min
Protein Expression reaction	• Incubator, constant temperature of +29°C • 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 Strep Beads NC3011-2	Cap color	Quantity	Storage instruction
Cell-free Core Reagent	Purple	5x160 µL	-80°C

Scale-up kit Strep Beads NC3011-2	Cap color	Quantity	Storage instruction
Wash Buffer	White	5x3 mL	-80°C
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 kit Strep Beads NC3011-2	Cap color	Quantity	Storage instruction
PDI/GSSG mix*	Green	1x175 µL	-80°C
TRXB1*	Green	1x175 µL	-80°C
DNAk mix*	Green	1x175 µL	-80°C
Zinc chloride	Green	1x175 µL	-80°C
Calcium chloride	Green	1x175 µL	-80°C
Manganese chloride	Green	1x175 µL	-80°C
Cofactor mix*	Green	1x175 µL	-80°C

Scale-up kit Strep Beads NC3011-2	Cap color	Quantity	Storage instruction
GSSG*	Green	1x175 µL	-80°C
3C protease*	Green	1x175 µL	-80°C

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

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
Selected Additive 1	15 µL	75µL
Selected Additive 2	15 µL	75µL
5 nM eGene DNA construct	50 µL	250µL

Reagents	Volume	
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:

- Give the vial(s) of Strep Beads a quick spin in a microcentrifuge to pellet the beads
- Pipette up and down to fully resuspend the beads.
- Transfer the beads to a 1.5 mL microcentrifuge tube.
- Place the tube(s) of Strep Beads for 1 minute on a magnetic particle collector to pellet the beads.
- Aspirate the storage buffer supernatant and discard.
- Remove the tube from the collector and resuspend the Strep Bead pellet with 400 µL Wash Buffer by pipetting up and down repeatedly.
- Repeat steps c, d and e for a total of 2 washes.
- Pellet the Strep Beads on the magnetic particle collector, aspirate and discard the supernatant.
- 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 17-19 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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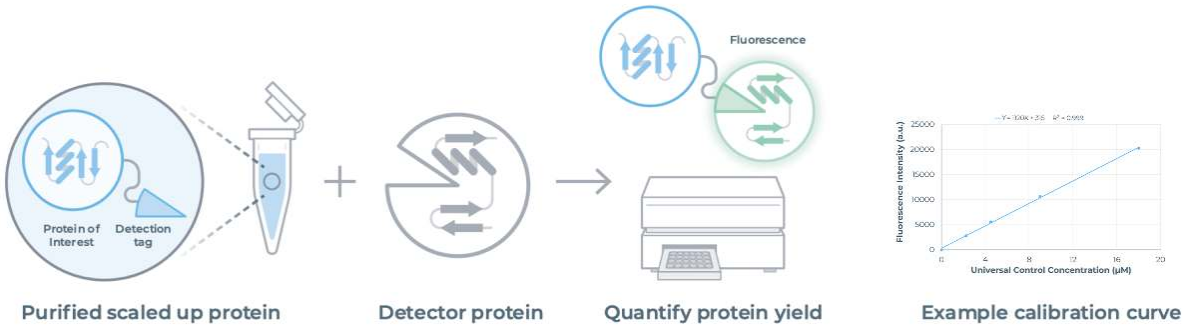
eProtein Quantification Kit Protocol

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).

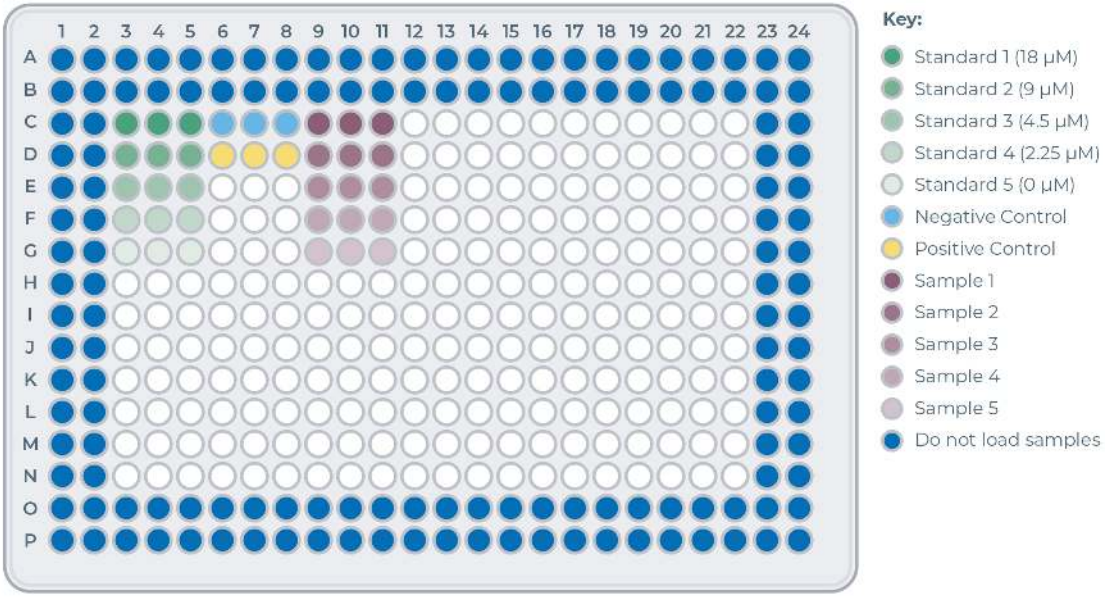
Preparation of Test Samples

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 pontrol 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.

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

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 • 0.15 M NaCl • 50 mM biotin • 0.05% nonionic detergent	• 0.1 M Tris-Cl • 0.15 M NaCl • 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 • 0.15 M NaCl • 50 mM biotin	• 0.1 M Tris-Cl • 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.

Videos

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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

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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.

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How to Set Up a Run

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