Linear eGene™ Preparation Kit - Solubility Tag Screen

eGene Prep Kit

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General information

The eGene™ Prep Kit NC3009 is designed to rapidly convert your synthesized DNA fragments into linear expression constructs that are immediately compatible with the eProtein Discovery™ system.

The kit has been designed for synthesized DNA fragments up to 3000 bp, and we recommend using gBlocks™ from our third party DNA vendor IDT.

In order to proceed with eGene preparation, which is outlined in this manual, you will need to have your synthesized gene fragments in hand.

When focused on obtaining certain targets, the Solubility Tag Screen allows you to explore a combination of POI variations & solubility tags to increase your chance of obtaining a soluble, active protein (Figure 1).

▹ Provides robust screen of constructs with different solubility tag options to increase chances of obtaining soluble protein
▹ 3, 4 or 6 proteins
▹ 4, 6 or 8 solubility tag options

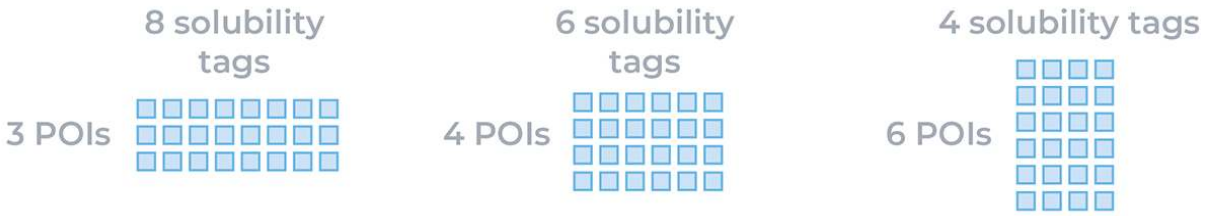


Figure 1: Possible combinations of POI variations & solubility tags

The eGene constructs are assembled based on a one pot, one step overlap extension PCR that primarily requires four key components: left megaprimer, right megaprimer, universal terminal primer pair and the DNA sequence of interest corresponding to the target protein of interest (POI) as shown in Figure 2.

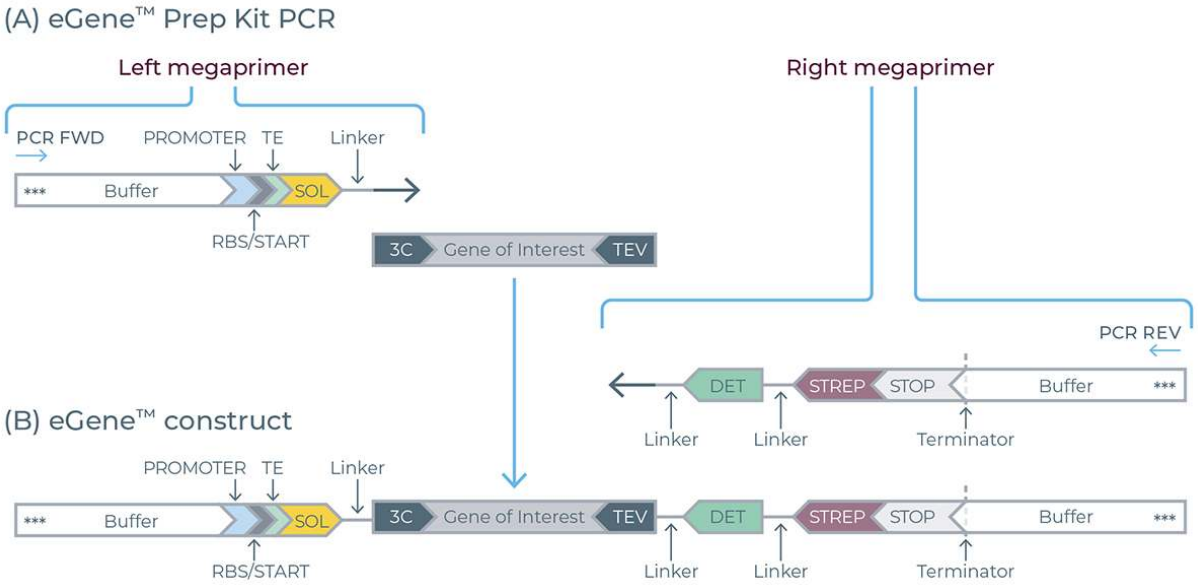


Figure 2. Description of the eGene components including megaprimers and resulting eGene construct. Ribosome binding site (RBS), Translation enhancer (TE), Solubility tag (SOL), 3C and TEV (protease binding sites), Detection tag (DET), streptactin based purification tag (STREP). The terminal primers used in the assembly process contain three phosphorothioate bonds (***) at their 5' ends.

The megaprimers are double stranded DNA molecules containing all the regulatory elements required for transcription and translation.

▹ The left megaprimer may contain a variety of solubility tags, while the right megaprimer always includes a GFP based detection tag (DET, 17 amino acids long, 1.95 kDa) along with a streptactin based purification tag (STREP).
▹ Importantly, the left megaprimer incorporates a 3C protease cleavage site at its 3' terminus while the right megaprimer incorporates TEV protease site at the 5' terminus providing the user flexibility to easily cleave off the additional tags from the purified proteins.
▹ The 3C-TEV protease cleavage sites also serve as the hybridization site in the overlap extension assembly reaction. Therefore, your gene of interest must be pre-adapted with 3C and TEV adaptor sequences at the 5' and 3' ends respectively, to be compatible with the eGene Prep Kit workflow.

During the overlap extension PCR, in the first 5-10 cycles multiple copies of the fully assembled products are formed. Thereafter, the universal primers take over to exponentially amplify the full-length assembled product.

Features and benefits

▹ Efficiency – expand one synthesized DNA fragment into up to eight different expression eGene constructs with different solubility tag options as shown in Table 1
▹ Robustness – individual constructs can yield 60 µL of up to 0.4 pmol DNA following eGene preparation
▹ Reliability – over 800 GOIs have been successfully converted into Grade 1 eGene constructs at the first attempt
▹ Speed – experiment set-up takes less than 30 minutes
▹ Accessibility – eluted, purified DNA is immediately ready-to-use in cell-free protein synthesis reactions

Contents

The eGene Prep kit - Solubility Tag Screen - NC3009 comes with the components listed in Table 1 and shown in Figure 3.

The components are supplied in a Nuclera branded box with a green stripe on the label, and must be used within 18 months from the manufacturing date indicated on the kit box.

Component	Volume	Cap Color	Storage Temperature	Tube reference number
P17-Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-01
CUSF-Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-02
FH8-Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-03
TRX-Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-04
ZZ-Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-05
SUMO-Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-06

Component	Volume	Cap Color	Storage Temperature	Tube reference number
SNUT-Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-07
Strep Primer Mix	135 µL	Blue	-80°C or -20°C	EG2-08
Re-amp Primer Mix	200 µL	Blue	-80°C or -20°C	EG2-10
10x Control Template	10 µL	Purple	-80°C or -20°C	EG2-11
Elution Buffer	18 mL	White	-80°C *	EG2-09

Table 1. List of components in the eGene Prep kit - Solubility Tag Screen - NC3009.

* at first use, make small 1.5 mL aliquots of the Elution Buffer, freeze at -80°C and treat as single use.

Figure 3: Components in the eGene Prep Kit - Solubility Tag Screen - NC3009

Contents Description

▹ **Primer Mix** formulations containing optimized concentrations of megaprimers corresponding to the left and right construct flanks, and universal forward and reverse terminal primers.

The eight megaprimer formulations allow the preparation of eight different linear expression constructs with or without varying N-terminal solubility tags (Table 2).

Component	N-term Sol	C-term DET	C-term PUR	eGene construct
P17-Strep Primer Mix	P17	Yes	Yes	P17-/POI/-DET-STREP
CUSF-Strep	CUSF	Yes	Yes	CUSF-/POI/-DET-

Component	N-term Sol	C-term DET	C-term PUR	eGene construct
Primer Mix				STREP
FH8-Strep Primer Mix	FH8	Yes	Yes	FH8-/POI/-DET-STREP
TRX-Strep Primer Mix	TRX	Yes	Yes	TRX-/POI/-DET-STREP
ZZ-Strep Primer Mix	ZZ	Yes	Yes	ZZ-/POI/-DET-STREP
SUMO-Strep Primer Mix	SUMO	Yes	Yes	SUMO-/POI/-DET-STREP
SNUT-Strep Primer Mix	SNUT	Yes	Yes	SNUT-/POI/-DET-STREP
Strep Primer Mix	-	Yes	Yes	-/POI/-DET-STREP

Table 2. Primer Mix components included in eGene Prep Kit NC3009.

▹ **Elution Buffer** required for the eProtein Discovery platform.

Note: Before first use, leave the Elution Buffer to thaw on ice for about 2 hours. Once open, make small 1.5 mL aliquots, store at -80°C and treat as single use.

▹ **Re-amp Primer Mix** for troubleshooting, or to produce larger quantities of DNA to scale up protein production (optional).

▹ **10x Control Template**, supplied as a PCR positive control to verify that the prepared formulation and thermocycling parameters are correct.

User supplied reagents / components (not included in the kit)

▹ Synthesised DNA fragments corresponding to the genes of interest (GOI) pre-adapted with 3C and TEV sequences on 5' and 3' ends, respectively. The GOI can be from 125 to 2955 bp long. We recommend gBlocks™ by IDT designed using the eProtein Discovery software.
▹ High fidelity PCR mastermix. We recommend using Platinum™ SuperFi II PCR Master Mixes or Phusion™ Plus PCR Master Mixes
▹ Nuclease free water (NFW)
▹ PCR purification kit (column- or bead-based methods)
▹ 1% (w/v) agarose gel
▹ DNA gel stain
▹ Loading buffer
▹ Electrophoresis running buffer
▹ DNA ladder

User supplied equipment

▹ Thermocycler
▹ Electrophoresis apparatus
▹ Gel doc or transilluminator
▹ Standard benchtop microcentrifuge
▹ Pipettes with disposable filter tips
▹ 0.2 mL thin-walled PCR tubes or 96-well PCR plate

eGene preparation workflow

IMPORTANT NOTE

To convert your synthesized DNA fragments into linear expression constructs successfully:

1. During eGene DNA construct (prep or re-synthesis) purification, eGene Elution Buffer must be used to ensure compatibility with eProtein Discovery Cartridges. Sub-dilutions must also be performed using the eGene Elution Buffer.
2. We recommend using filter tips throughout the protocol to prevent cross-contamination.
3. When outsourcing linear DNA fragments, the length of the DNA of interest (excluding Nuclera adaptor sequences) should be between 125 and 2955 base pairs.

Preparation of the template DNA

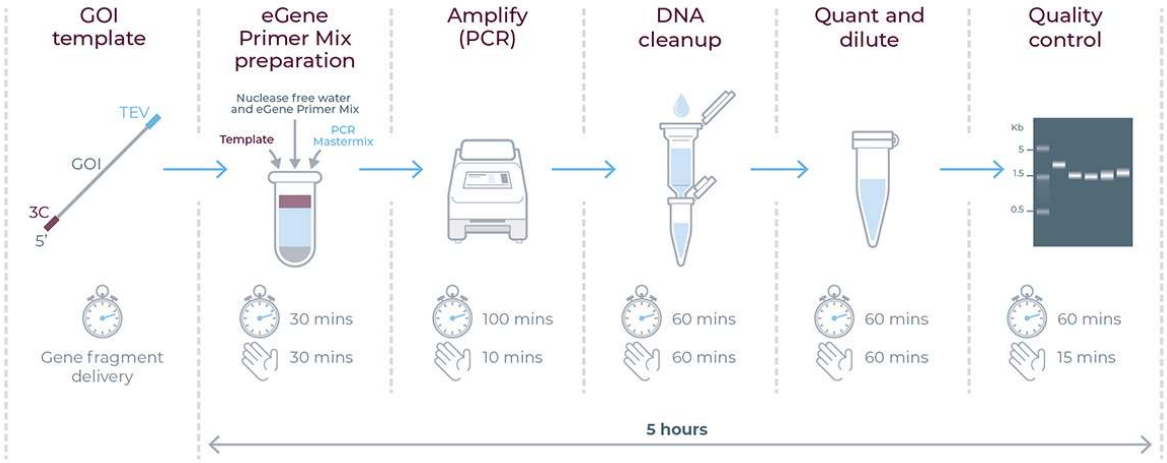


Figure 4. eGene workflow summary - timings applicable for generating eGene constructs using the eGene Prep Kit. For each step of the workflow, the clock indicates the total

experiment time and the hand indicates the total hands-on time.

> NOTE
>
> If template DNA are gene fragments (gBlock™) supplied by IDT **in tube**, follow the steps 1 to 6 to dilute it
>
> If template DNA are gene fragments (gBlock™) supplied by IDT **in a 96-well plate** go forward to *Preparation of the template solutions* section, as DNA fragments are already resuspended.

1. Before opening the tube, spin it down in a microcentrifuge for 5 seconds to ensure all the lyophilized DNA fragment from IDT is at the bottom of the tube.
   The lyophilized DNA fragment pellet can be statically charged and adhere to the tube wall or cap, resulting in loss of material.
2. Add a suitable buffer such as 0.1X TE or nuclease-free water, to reach a final concentration of 10 ng/µL. For example, if the tube has 1000 ng of lyophilized product, add 100 µL of molecular grade water, or a buffer to resuspend it.
3. Vortex briefly to resuspend DNA fragments into solution.
4. Either leave the solution at 4°C overnight or incubate at 50°C for 15–20 min. Both methods will ensure that the entire pellet will be resuspended in the buffer or nuclease-free water.
5. Briefly vortex and centrifuge.
6. Convert DNA concentration to molarity using the following formula:

$$GOI(nM) = \frac{Concentration\ (ng/\mu L)}{[Length(bp) \times 617.96\ g/mol/bp]\ +\ 36.04\ g/mol} \times 10^6$$

Preparation of the template solutions

1. **PCR control template:** In a 200 µL thin-walled PCR tube, add 1 µl of 10x Control Template to 9 µL of nuclease-free water to obtain a Control Template solution at 2 nM and keep on ice.
2. **Gene of interest template:** Prepare a 2 nM GOI template solution by diluting the required volume of GOI (calculated using the formula below) in 10 µL of nuclease-free water. Keep the solution on ice

$$Volume\ GOI(\mu L) = \frac{2(Target\ Molar\ Concentration\ (nM)) \times 10(NFW\ (\mu L))}{Obtained\ Molar\ Concentration(nM) - 2(Target\ Molar\ Concentration(nM))}$$

Example: For a 2159 bp gene of interest obtained at 10 ng/µL, which corresponds to 7.5 nM, add 3.6 µL of DNA template to 10 µL of nuclease-free water.

Preparation of the PCR reaction mix

The assembly reaction consists of adding the template DNA to the reaction mix containing a Primer Mix and a 2x polymerase mastermix (Figure 5).

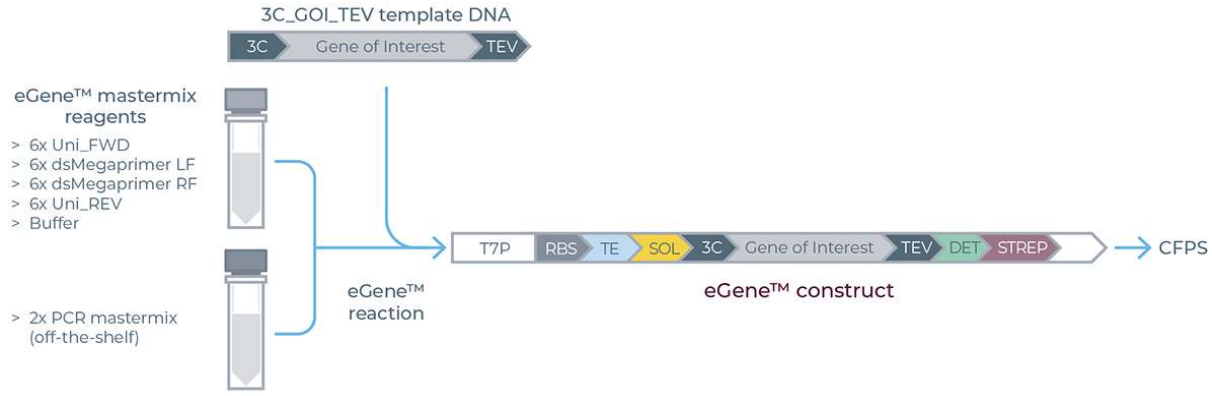


Figure 5. Overview of the eGene Prep Kit assembly reaction set-up. T7 promoter (T7p), Ribosome Binding Site (RBS), translation enhancer (TE), solubility tag (SOL), 3C (human

rhinovirus) protease cleavage site (3C), TEV (Tobacco etch virus) protease cleavage site (TEV), detection tag (DET), Strep purification tag (STREP).

1. Thaw on ice each of the individual vials containing the Primer Mixes (see details in Table 2). After use, refreeze any unused material. The number of reactions varies depending on the experimental format planned to be applied with the eProtein Discovery instrument:
   ▹ 3 proteins of interest with 8 eGene constructs for each protein
   ▹ 4 proteins of interest with 6 eGene constructs for each protein
   ▹ 6 proteins of interest with 4 eGene constructs for each protein

2. Quick spin down the tube for 10 seconds using a microcentrifuge.

3. To facilitate the work you can prepare a mastermix for each GOI by adding nuclease free water, 2x PCR mastermix and GOI. Aliquot 50 µL of the mastermix into PCR strip tubes/wells followed by addition of 10 µL of the eGene Primer Mix into the respective tubes/wells. Prepare a negative control and a positive control independently.

Note: Use randomly one of the 8 Primer Mix reagents provided in the kit in the two wells or PCR tubes for the positive and negative controls

Reagents	Reaction Volume
Nuclease free water (NFW)	19 µL
2X PCR mastermix (high fidelity PCR mastermix, user supplied)	30 µL
Gene of interest at 2 nM, or water (negative control), or	1 µL

Reagents	Reaction Volume
Control Template (positive control)	
Primer Mix	10 µL
Final volume (maximum recommended volume per reaction)	60 µL

Table 3. Preparation of the PCR reactions.

PCR assembly

1. Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.
2. Place your samples in a thermal block cycler and perform PCR using the parameters in Table 4.

PCR steps	Temperature	Time	Number of cycles
Pre-Incubation	98°C	30 sec	1
Denaturation	98°C	10 sec	27
Annealing	60°C	20 sec	
Elongation	72°C*	30 sec per kb**	

PCR steps	Temperature	Time	Number of cycles
Final Elongation	72°C*	2 minutes	1

Table 4. Thermocycler parameters.

* Elongation temperature: to be based on manufacturer's recommendation.

** Elongation time: to be based on manufacturer's recommendation but should not be less than 30 sec per kb of the final eGene construct.

Evaluation of the amplified DNA by agarose gel electrophoresis

1. Prepare a 1% (w/v) agarose gel with a DNA gel stain in 1x Tris acetate EDTA (TAE) or 1x Tris-borate-EDTA (TBE) buffer.
2. Using new wells or new PCR tubes, prepare 12 µL samples for loading on the gel as detailed in Table 5.

6x gel loading dye	Nuclease free water	eGene DNA construct
2 µL	8 µL	2 µL

Table 5. Sample preparation for agarose gel electrophoresis.

Note: a bulk solution of ready-to-use 1x loading buffer can be made and stored at room temperature or +4°C.

3. Load 10 µL of PCR products and control on 1% agarose gel along with an appropriate DNA ladder (1 kb).

If the purity of the eGene constructs is low with the presence of one or more extra band(s) on the gel, there is a risk to obtain the protein of interest with

a significant amount of impurities.

Troubleshooting: For eGene constructs containing more than one band, a band-stab and reamplification is required using the Re-amp Primer Mix provided in the eGene Prep kit. The band-stab and reamplification protocols can be found in the Troubleshooting section of this document.

PCR purification

The PCR reactions must be cleaned using the DNA purification method of your choice, either column or bead-based methods. Gel extraction is not recommended as it might result in significant product yield loss.

> IMPORTANT NOTE
>
> The samples must be eluted using the Elution Buffer (50 µL) supplied in the eGene Prep kit to make them compatible with the eProtein Discovery cartridge. The eGene Elution Buffer is a HEPES buffer, pH 8.0, containing a surfactant.

DNA quantification

1. It is recommended to carry out DNA quantification (ng/µL) using fluorescent dye-based methods, not spectrophotometric methods.

2. Determine the length of the eGene using the calculator available on the eProtein Discovery Software or the formula below and Table 6.

Note: the length of the GOI should not include the length of the 3C and TEV sequences.
Note: the length of the Control Template supplied in the kit is 660 bp, 3C and TEV sequence lengths not included.

eGene Length (bp) = GOI Length (bp) + Primer Mix Length (bp)

Primer Mix	Total length of the flanks (bp)
P17-Strep	1134
CUSF-Strep	1302
FH8-Strep	1239
TRX-Strep	1359
ZZ-Strep	1383
SUMO-Strep	1341
SNUT-Strep	1476
-Strep only	990

Table 6. Total lengths of the N-terminal and C-terminal flanks to add to the gene of interest to determine the total length of the eGene constructs

3. Calculate the molar concentration (nM) of the eGene constructs using the calculator available on the eProtein Discovery Software or the following formula:

$$eGene\ (nM) = \frac{Concentration\ (ng/\mu L)}{[Length(bp) \times 617.96\ g/mol/bp]\ +\ 36.04\ g/mol} \times 10^6$$

Example: the molar concentration of a 1415 bp long eGene at 60 ng/µL is 68.6 nM

$$\frac{60}{(1415 \times 617.96) + 36.04} \times 10^6 = 68.6nM$$

Note: If the DNA concentration is lower than 5 nM, please refer to the reamplification protocol in the troubleshooting section to generate more DNA.

eGene constructs normalization

To be compatible with the eProtein Discovery platform, all eGene constructs must be normalized to 5 nM using the Elution Buffer provided in the eGene Prep Kit.

60 µL of an eGene at 5 nM is sufficient for one experimental run on the eProtein Discovery instrument and a 200 µL scale-up experiment.

Determine the volume of eGene constructs to add to 60 µL of eGene Elution Buffer using the following formula:

$$Volume\ eGene(\mu L) = \frac{5(Target\ Molar\ Concentration\ in\ nM) \times 60(eGene\ Elution\ Buffer\ (\mu L))}{Obtained\ Molar\ Concentration\ (nM)\ -5\ (Target\ Molar\ Concentration\ (nM))}$$

Example: For a 2589 bp eGene obtained at 40 ng/µL, which corresponds to 25 nM, add 15 µL of DNA sample to 60 µL of eGene Elution Buffer.

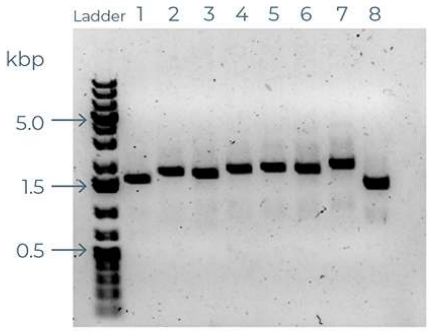
Final gel QC

It is recommended to run 2 µL of the samples on a 1% agarose gel post PCR clean-up.

Note: This step is optional if you already evaluated the amplified DNA by electrophoresis after the PCR assembly.

eGene constructs must be of high purity since the input DNA sequence is transcribed in the Cell-free Protein Synthesis reaction and the subsequent mRNA is translated to the final protein of interest.

The quality of DNA generated using the eGene Prep Kit - Solubility Tag Screen (NC3009) is illustrated in Figure 6 (agarose gel) and 7 (electropherogram), showing a comparison of the PCR products from incorporating different solubility tags using the standard solubility tag screen, while keeping the 'Gene of Interest' (GOI) constant.



Lane	Description	Lane	Description
1	P17-GOI-DET-STREP	5	ZZ-GOI-DET-STREP
2	CUSF-GOI-DET-STREP	6	SUMO-GOI-DET-STREP
3	FH8-GOI-DET-STREP	7	SNUT-GOI-DET-STREP
4	TRX-GOI-DET-STREP	8	GOI-DET-STREP

Figure 6: Example of an agarose gel illustrating the DNA bands generated from PCR products of the GOI CALM1 (UniProt ID P0DP23) expanded with different solubility tags.

Lane	Description	Lane	Description
1	P17-GOI-DET-STREP	5	ZZ-GOI-DET-STREP
2	CUSF-GOI-DET-STREP	6	SUMO-GOI-DET-STREP
3	FH8-GOI-DET-STREP	7	SNUT-GOI-DET-STREP
4	TRX-GOI-DET-STREP	8	GOI-DET-STREP

Figure 7. Example of a capillary electrophoresis generated electropherogram illustrating the DNA bands generated from PCR products of the GOI rpsA (UniProt ID P0AG67) expanded with different solubility tags

If the purity of the eGene constructs is low with the presence of one or more extra band(s) on the gel, there is a risk to obtain the protein of interest with a significant amount of impurities.

Troubleshooting: For eGene constructs containing more than one band, a band-stab and reamplification is required using the Re-amp Primer Mix provided in the eGene Prep kit. The band-stab and reamplification protocols can be found in the Troubleshooting section of this document.

Storage of the eGene constructs

Purified and normalized eGene constructs can be stored at -20°C short term (days) or -80°C for long term storage.

eGene Optimization / Troubleshooting

1. Band-stab protocol

Note: Always wear UV safety glasses when working with a transilluminator.

Overview: Band-stab is suggested to obtain pure eGene constructs directly from the eGene PCR assembly using the eGene Prep Kit. An example of a band-stabbed gel is in Figure 8.

It is a simple method to obtain single bands as compared to gel purification techniques which requires excision and purification of the DNA fragment from the agarose gel.

The method described in this section is adapted from the publication by Bjourson and Cooper (*Bjourson AJ, Cooper JE. Band-stab PCR: a simple technique for the purification of individual PCR products. Nucleic Acids Res. 1992 Sep 11; 20(17): 4675*).

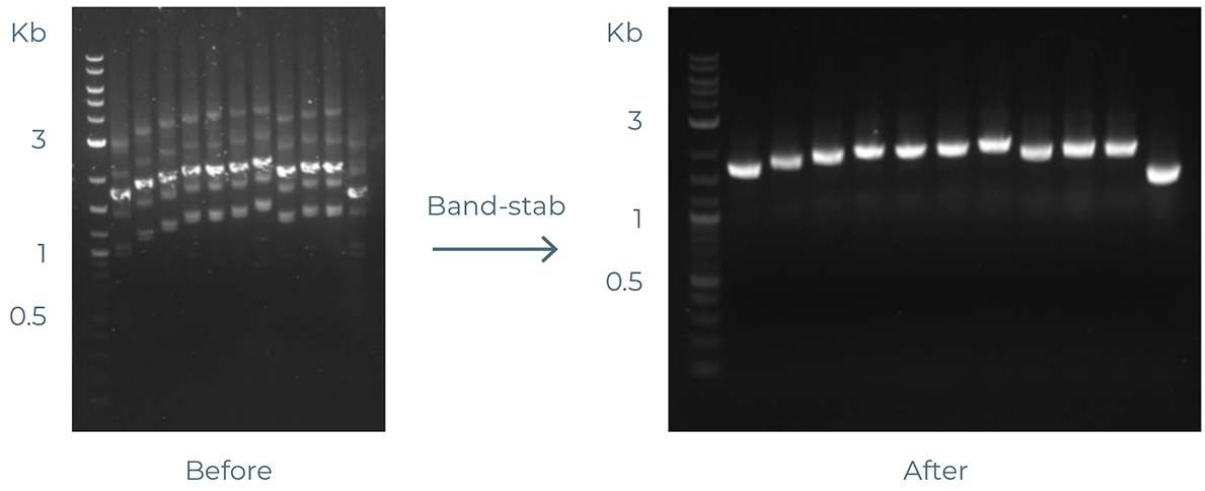


Figure 8: Example of band-stabbed gel.

1. Run the eGene constructs showing non-specific bands on a 1 % (w/v) agarose gel, or use the gel made for the final gel QC step.
2. Load 2 µL of purified product on the gel.
3. Set-up the electrophoresis apparatus for 45 minutes at 120 Volts, or until the desired band is well separated.
4. While the electrophoresis is running, prepare the reamplification PCR reaction as described in Section 2. Reamplification.
5. When the electrophoresis run is complete, take the gel from the tray and take a picture using an imaging apparatus and locate where your desired bands are.
6. Place the gel on the transilluminator and use the blue light setting.
7. Locate your band of interest and take a P10 pipette with a P10 filter tip attached.
8. Hold the pipette vertically on top of the desired band and stab the band three times per PCR reaction.
9. Transfer the pipette tip into the PCR reamplification reaction mix. It is recommended to pipette up and down three times to release all the material from the pipette tip into the PCR reaction solution.

Note: avoid transferring pieces of gel into the PCR reaction mix as they may inhibit the amplification process

2. Reamplification

General information

The reamplification employs the same principles of a regular PCR reaction. This method allows synthesizing more of a eGene construct, either collected from a band-stab protocol, or in the case the quantity is not sufficient for a use on the eProtein Discovery instrument or for a scale-up

experiment.

Notes:
▹ Always use an original eGene assembly as template for PCR reamplification. It is not recommended to use a reamplified product as a template as this would increase the risk of mutations over the subsequent generations.
▹ The minimum number of reactions that should be prepared is 3 x 50 µL:

- The first reaction is with the template eGene construct
- The second reaction serves as a no template control.
- The third reaction is prepared as a spare reaction. Due to the different viscosities and temperatures of the reagents, the pipetting volumes can be inaccurate leading to shortage of reaction solution.

▹ If re-amplifying more than one construct, it is recommended to prepare a sufficient reaction solution containing all the components except the template that are subsequently added to the respective wells or tubes.

▹ Reamplification product lengths are 40 bp shorter than original eGene constructs due to the use of a nested primer pair to synthesize them.

Preparation of the eGene template DNA to be reamplified

1. Prepare a 10 µL solution of the eGene template normalized to 2 nM and keep on ice. Example: For a 2159 bp gene of interest obtained at 10 ng/µL, which corresponds to 7.5 nM, add 3.6 µL of DNA template to 10 µL of nuclease-free water.

Preparation of the reamplification PCR reaction mix

1. Thaw 2x PCR Mastermix and Re-amp Primer Mix at room temperature and spin down tube for 10 seconds at 1000 g.

2. For each reamplification PCR reaction, add the components listed in

Table 7 and keep the tubes or the PCR plate on ice

Note: An additional well or PCR tube should be used as negative control, using nuclease free water instead of the eGene.

Reagent	Reaction volume
Nuclease free water (NFW)	21.5 µL
2X PCR mastermix (high fidelity PCR mastermix, user supplied)	25 µL
Re-amp Primer Mix	2.5 µL
eGene DNA template, or nuclease free water	1 µL
Final Volume	50 µL

Table 7. Preparation of the PCR reaction solutions.

Reamplification PCR reaction

1. Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.
2. Place your samples in a thermal block cycler and perform PCR using the parameters in Table 8.

PCR steps	Temperature	Time	Number of cycles
Pre-Incubation	98°C	30 sec	1

PCR steps	Temperature	Time	Number of cycles
Denaturation	98°C	10 sec	19
Annealing	60°C	20 sec	
Elongation	72°C*	30 sec per kb**	
Final Elongation	72°C*	2 minutes	1

Table 8. Thermocycler parameters

* Elongation temperature: to be based on manufacturer's recommendation.
** Elongation time: to be based on manufacturer's recommendation but should not be less than 30 seconds per kb of the final eGene construct.

3. To evaluate, purify, quantify and normalize the obtained re-amplified eGene constructs, follow the same procedure as from section 'Evaluation of the amplified DNA by agarose gel electrophoresis'.

Linear eGene™ Preparation Kit - Flexivariant Screen

eGene Prep Kit

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General information

The eGene™ Prep Kit NC3008 is designed to rapidly convert your synthesized DNA fragments into linear expression constructs that are immediately compatible with eProtein Discovery™ Cell Free Protein Synthesis (CFPS).

The kit has been designed for synthesized DNA fragments up to 3000 bp, and we recommend using gBlocks™ from IDT.

In order to proceed with eGene preparation, which is outlined in this manual, you will need to have your synthesized gene fragments in hand.

The FlexiVariant Screen adds Detection Tag and Purification Tag to the C-terminus of your DNA constructs. It is recommended for use to evaluate solubility and yield between full length target protein and various variants such as mutations, truncations, isoforms and homologs, or simply screen 24 different protein-of-interest (Figure 1).

The FlexiVariant Screen allows you to maximize target screening on the

same cartridge without the use of solubility tags:

▹ Use when solubility tag is not tolerated or when the removal of solubility tag is not favored
▹ Screen 24 different proteins to get a quick idea of obtainability and narrow down focus
▹ Explore full length and 23 combinations of truncations / isoforms / homologs to compare expression yields
▹ Screen 24 different mutation sites

Figure 1. Schematic concept of the eGene Prep Kit - FlexiVariant - NC3008

The eGene constructs are assembled based on a one pot, one step overlap extension PCR that primarily requires four key components: left megaprimer, right megaprimer, universal terminal primer pair and the gene sequence of interest corresponding to the target protein of interest (POI) as shown in Figure 2.

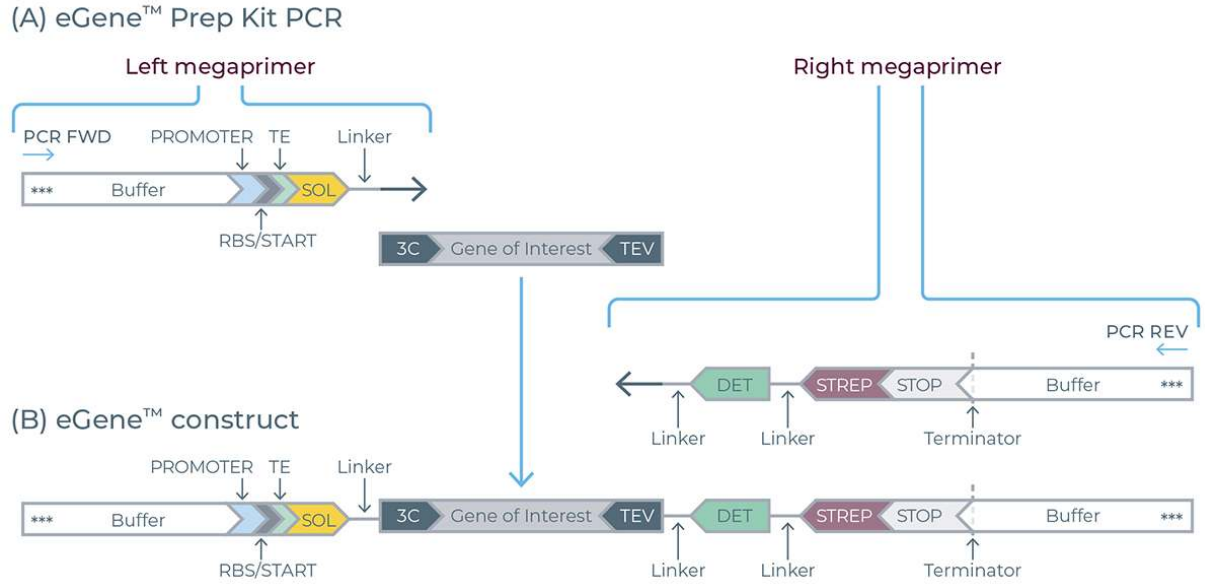


Figure 2. Description of the eGene components including megaprimers and resulting eGene construct. Ribosome binding site (RBS), Translation enhancer (TE), 3C and TEV (protease binding sites), Detection tag (DET), streptactin based purification tag (STREP). The terminal primers used in the assembly process contain three phosphorothioate bonds (***) at their 5' ends.

The megaprimers are double stranded DNA molecules containing all the regulatory elements required for transcription and translation.
▹ The right megaprimer always includes a GFP based detection tag (DET, 17 amino acids long, 1.95 kDa) along with a streptactin based purification tag (STREP).
▹ Importantly, the left megaprimer incorporates a 3C protease cleavage site at its 3' terminus while the right megaprimer incorporates TEV protease site at the 5' terminus providing the user flexibility to easily cleave off the additional tags from the purified proteins.
▹ The 3C-TEV protease cleavage sites also serve as the hybridization site in the overlap extension assembly reaction. Therefore, your gene of interest must be pre-adapted with 3C and TEV adaptor sequences at the 5' and 3' ends respectively, to be compatible with the eGene Prep Kit workflow.

During the overlap extension PCR, in the first 5-10 cycles multiple copies of the fully assembled products are formed. Thereafter, the universal primers take over to exponentially amplify the full-length assembled product.

Features and benefits

▹ High-throughput – allows you to maximize construct screening on the same cartridge by minimizing the use of fusion tags
▹ Robustness – individual constructs can yield 60 µL of up to 0.4 pmol DNA following eGene preparation
▹ Reliability – over 800 GOIs have been successfully converted into Grade 1 eGene constructs at the first attempt

▹ **Speed** – experiment set-up takes less than 30 minutes
▹ **Accessibility** – eluted, purified DNA is immediately ready-to-use in cell-free protein synthesis reactions

Contents

The eGene Prep kit - Solubility Tag Screen - NC3009 comes with the components listed in Table 1 and shown in Figure 3.

The components are supplied in a Nuclera branded box with a green stripe on the label, and must be used within 18 months from the manufacturing date indicated on the kit box.

Component	Volume	Cap Color	Storage Temperature	Tube reference number
Strep Primer Mix	1.1 mL	Blue	-80°C or -20°C	EG2-08
Re-amp Primer Mix	200 µL	Blue	-80°C or -20°C	EG2-10
10x Control Template	10 µL	Purple	-80°C or -20°C	EG2-11
Elution Buffer	18 mL	White	-80°C *	EG2-09

Table 1. List of components in the eGene Prep kit - Solubility Tag Screen - NC3008.
* at first use, make small 1.5 mL aliquots of the Elution Buffer, freeze at -80°C and treat as single use.

Contents description

▹ Primer Mix formulations containing predefined concentrations of megaprimers corresponding to the left and right construct flanks, and universal terminal primers. The megaprimer formulation allows the preparation of linear expression constructs with no N-terminal solubility tag (Table 2).

Component	N-term Sol	C-term DET	C-term PUR	eGene construct
-Strep Primer Mix	-	Yes	Yes	-POI-DET-STREP

Table 2: Primer Mix components included in eGene Prep Kit NC3008.

▹ Elution Buffer required for the eProtein Discovery platform.

Note: Before first use, leave the Elution Buffer to thaw on ice for about 2 hours. Once open, make small 1.5 mL aliquots, store at -80°C and treat as single use.

▹ Re-amp Primer Mix for troubleshooting, or to produce larger quantities of DNA to scale up protein production (optional).

▹ 10x Control Template, supplied as a PCR positive control to verify that the prepared formulation and thermocycling parameters are correct.

User supplied reagents / components (not included in the kit)

▹ Synthesised DNA fragments corresponding to the genes of interest (GOI) pre-adapted with 3C and TEV sequences on 5' and 3' ends, respectively. The GOI can be from 125 to 2955 bp long. We recommend gBlocks™ by IDT designed using the eProtein Discovery software.
▹ High fidelity PCR mastermix. We recommend using: Platinum™ SuperFi II PCR Master Mixes or Phusion™ Plus PCR Master Mixes
▹ Nuclease free water (NFW)
▹ PCR purification kit (column- or bead-based methods)
▹ 1% (w/v) agarose gel
▹ DNA gel stain
▹ Loading buffer
▹ Electrophoresis running buffer
▹ DNA ladder

User supplied equipment

▹ Thermocycler
▹ Electrophoresis apparatus
▹ Gel doc or transilluminator
▹ Standard benchtop microcentrifuge
▹ Pipettes with disposable filter tips
▹ 0.2 mL thin-walled PCR tubes or 96-well PCR plate

eGene preparation workflow

IMPORTANT NOTE

To convert your synthesized DNA fragments into linear expression constructs successfully:

1. During eGene DNA construct (prep or re-synthesis) purification, eGene Elution Buffer must be used to ensure compatibility with eProtein Discovery Cartridges. Sub-dilutions must also be performed using the eGene Elution Buffer.
2. We recommend using filter tips throughout the protocol to prevent cross-contamination.
3. When outsourcing linear DNA fragments, the length of the DNA of interest (excluding Nuclera adaptor sequences) should be between 125 and 2955 base pairs.

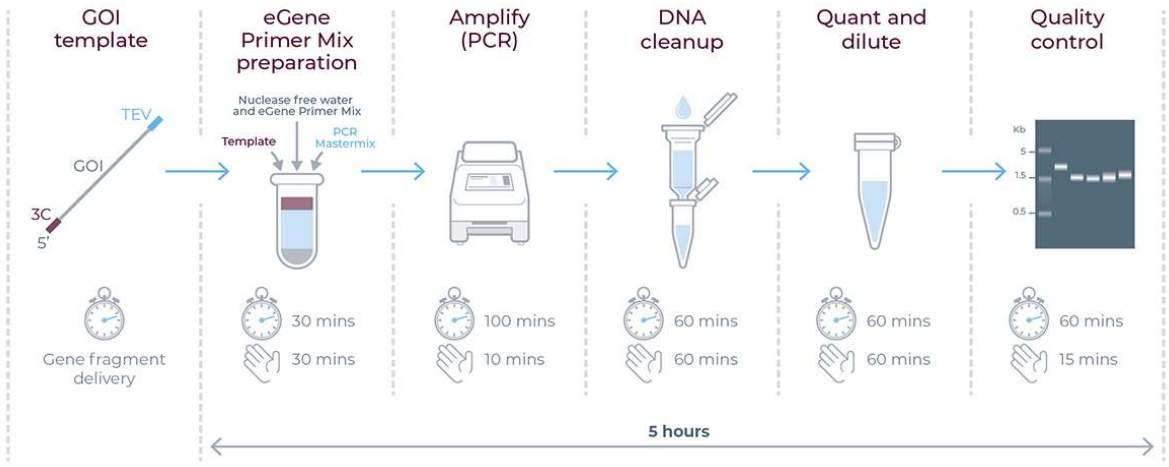


Figure 3. eGene workflow summary - timings applicable for generating eGene constructs using the eGene Prep Kit. For each step of the workflow, the clock indicates the total experiment time and the hand indicates the total hands-on time.

Preparation of the template DNA

> NOTE
>
> If template DNA are gene fragments (gBlock™) supplied by IDT **in tube**, follow the steps 1 to 6 to dilute it.
>
> If template DNA are gene fragments (gBlock™) supplied by IDT **in a 96-well plate** go forward to *Preparation of the template solutions* section, as DNA fragments are already resuspended.

1. Before opening the tube, spin it down in a microcentrifuge for 5 seconds to ensure all the lyophilized DNA fragment from IDT is at the bottom of the tube. The lyophilized DNA fragment pellet can be statically charged and adhere to the tube wall or cap, resulting in loss of material.
2. Add a suitable buffer such as 0.1X TE or nuclease-free water, to reach a final concentration of 10 ng/µL. For example, if the tube has 1000 ng of lyophilized product, add 100 µL of molecular grade water, or a buffer to resuspend it.
3. Vortex briefly to resuspend DNA fragments into solution.
4. Either leave the solution at +4°C overnight or incubate at 50°C for 15–20 min. Both methods will ensure that the entire pellet will be resuspended in the buffer or nuclease-free water.
5. Briefly vortex and centrifuge.
6. Convert DNA concentration to molarity using the following formula:

$$GOI(nM) = \frac{Concentration\ (ng/\mu L)}{[Length(bp) \times 617.96\ g/mol/bp]\ +\ 36.04\ g/mol} \times 10^6$$

Preparation of the template solutions

1. **PCR control template:** In a 200 µL thin-walled PCR tube, add 1 µl of 10x Control Template to 9 µL of nuclease-free (NFW) water to obtain a Control Template solution at 2 nM and keep on ice.
2. **Gene of interest template:** Prepare a 2 nM GOI template solution by diluting the required volume of GOI (calculated using the formula below) in 10 µL of nuclease-free water. Keep the solution on ice

$$Volume\ GOI(\mu L) = \frac{2(Target\ Molar\ Concentration\ (nM)) \times 10(NFW\ (\mu L))}{Obtained\ Molar\ Concentration(nM) - 2(Target\ Molar\ Concentration(nM))}$$

Example: For a 2159 bp gene of interest obtained at 10 ng/µL, which corresponds to 7.5 nM, add 3.6 µL of DNA template to 10 µL of nuclease-free water (NFW).

Preparation of the PCR reaction mix

The assembly reaction consists of adding the template DNA to the reaction mix containing a Primer Mix and a 2x polymerase mastermix (Figure 4).

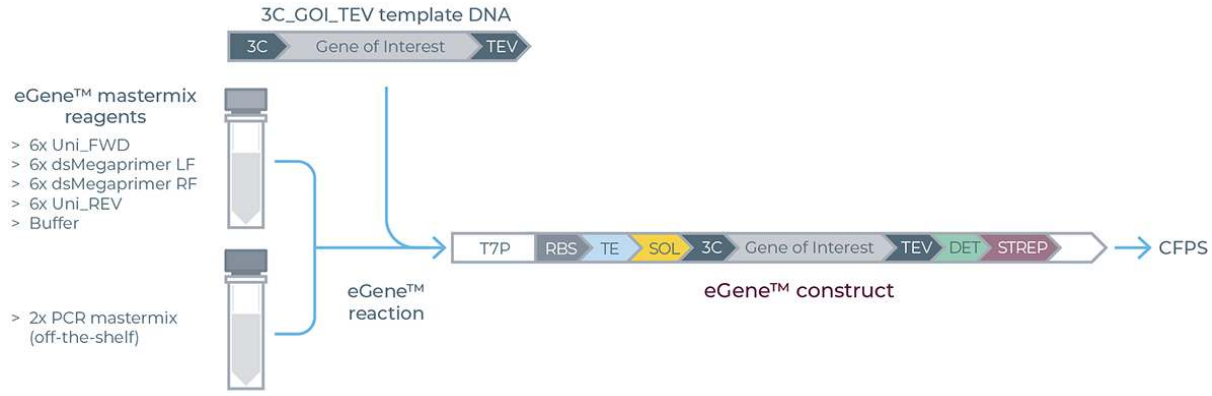


Figure 4. Overview of the eGene Prep Kit assembly reaction set-up. T7 promoter (T7p), Ribosome Binding Site (RBS), translation enhancer (TE), solubility tag (SOL), 3C (human

rhinovirus) protease cleavage site (3C), TEV (Tobacco etch virus) protease cleavage site (TEV), detection tag (DET), Strep purification tag (STREP).

1. Thaw the vial containing the Primer Mix. After use, refreeze any unused material.
2. Quick spin down the tube for 10 seconds using a microcentrifuge.
3. Prepare the mastermix for PCR reaction

Reagents	Reaction Volume
Nuclease free water (NFW)	19 µL
2X PCR mastermix (high fidelity PCR mastermix, user supplied)	30 µL
Primer Mix	10 µL
Gene of interest at 2 nM, or water (negative control), or Control Template (positive control)	1 µL
Final volume (maximum recommended volume per reaction)	60 µL

Table 3: Preparation of the PCR reactions.

▹ Prepare a mastermix for 27 reactions by mixing the components indicated in Table 3.

▹ Dispense 59 µL of mastermix prepared as outline in table 3 into each tube labelled 1-26.

▹ Dispense 1 µL of 2 nM template GOI directly into tubes labelled 1- 24.

▹ Dispense 1 uL of 2 nM Control Template in tube 25; this will be your Positive Control.

▹ Dispense 1 µL of NFW in tube 26; this will be your no template control.

PCR assembly

1. Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.
2. Place your samples in a thermal block cycler and perform PCR using the parameters in Table 4.

PCR steps	Temperature	Time	Number of cycles
Pre-Incubation	98°C	30 sec	1
Denaturation	98°C	10 sec	27
Annealing	60°C	20 sec	
Elongation	72°C*	30 sec per kb**	
Final Elongation	72°C*	2 minutes	1

Table 4. Thermocycler parameters.

* Elongation temperature: to be based on manufacturer's recommendation.

** Elongation time: to be based on manufacturer's recommendation but should not be less than 30 seconds per kb of the final eGene construct.

Evaluation of the amplified DNA by agarose gel electrophoresis

1. Prepare a 1% (w/v) agarose gel with a DNA gel stain in 1x Tris acetate EDTA (TAE) or 1x Tris-borate-EDTA (TBE) buffer.

2. Using new wells or new PCR tubes, prepare 12 µL samples for loading on the gel as detailed in Table 5.

6x gel loading dye	Nuclease free water	eGene DNA construct
2 µL	8 µL	2 µL

Table 5. Sample preparation for agarose gel electrophoresis.

Note: a bulk solution of ready-to-use 1x loading buffer can be made and stored at room temperature or 4°C.

3. Load 10 µL of PCR products and control on 1% agarose gel along with an appropriate DNA ladder (1 kb).

If the purity of the eGene constructs is low with the presence of one or more extra band(s) on the gel, there is a risk to obtain the protein of interest with a significant amount of impurities.

Troubleshooting: For eGene constructs containing more than one band, a band-stab and reamplification is required using the Re-amp Primer Mix provided in the eGene Prep kit. The band-stab and reamplification protocols can be found in the Troubleshooting section of this document.

PCR purification

The PCR reactions must be cleaned using the DNA purification method of your choice, either column or bead-based methods. Gel extraction is not recommended as it might result in significant product yield loss.

> IMPORTANT NOTE

> The samples must be eluted using the Elution Buffer (50 µL) supplied in the eGene Prep kit to make them compatible with the eProtein Discovery cartridge. The eGene Elution Buffer is a HEPES buffer, pH 8.0, containing a surfactant.

DNA quantification

1. It is recommended to carry out DNA quantification (ng/µL) using fluorescent dye-based methods, not spectrophotometric methods.

2. Determine the length of the eGene using the calculator available on the eProtein Discovery Software or the formula below and Table 6.

Note: the length of the GOI should not include the length of the 3C and TEV sequences.

Note: the length of the Control Template supplied in the kit is 660 bp, 3C and TEV sequence lengths not included.

eGene Length (bp) = GOI Length (bp) + Primer Mix Length (bp)

Primer Mix	Total length of the flanks (bp)
-Strep only	990

Table 6. Total lengths of the N-terminal and C-terminal flanks to add to the gene of interest to determine the total length of the eGene constructs

3. Calculate the molar concentration (nM) of the eGene constructs using the calculator available on the eProtein Discovery Software or the following formula:

$$eGene\ (nM) = \frac{Concentration\ (ng/\mu L)}{[Length(bp) \times 617.96\ g/mol/bp]\ +\ 36.04\ g/mol} \times 10^6$$

Example: the molar concentration of a 1415 bp long eGene at 60 ng/µL is 68.6 nM

$$\frac{60}{(1415 \times 617.96) + 36.04} \times 10^6 = 68.6nM$$

Note: If the DNA concentration is lower than 5 nM, please refer to the reamplification protocol in the troubleshooting section to generate more DNA.

eGene constructs normalization

To be compatible with the eProtein Discovery platform, all eGene constructs must be normalized to 5 nM using the Elution Buffer provided in the eGene Prep Kit.

60 µL of an eGene at 5 nM is sufficient for one experimental run on the eProtein Discovery instrument and a 200 µL scale-up experiment.

Determine the volume of eGene constructs to add to 60 µL of eGene Elution Buffer using the following formula:

$$Volume\ eGene(\mu L) = \frac{5(Target\ Molar\ Concentration\ in\ nM) \times 60(eGene\ Elution\ Buffer\ (\mu L))}{Obtained\ Molar\ Concentration\ (nM)\ -5\ (Target\ Molar\ Concentration\ (nM))}$$

Example: For a 2589 bp eGene obtained at 40 ng/µL, which corresponds to 25 nM, add 15 µL of DNA sample to 60 µL of eGene Elution Buffer.

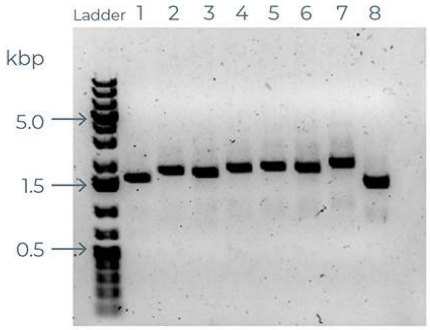
Final gel QC

It is recommended to run 2 µL of the samples on a 1% agarose gel post PCR clean-up.

Note: This step is optional if you already evaluated the amplified DNA by electrophoresis after the PCR assembly.

eGene constructs must be of high purity since the input DNA sequence is transcribed in the Cell-free Protein Synthesis reaction and the subsequent mRNA is translated to the final protein of interest.

The quality of DNA generated using the eGene Prep Kit - Solubility Tag Screen (NC3009) is illustrated in Figure 5 (agarose gel) and 6 (electropherogram), showing a comparison of the PCR products from incorporating different solubility tags using the standard solubility tag screen, while keeping the 'Gene of Interest' (GOI) constant.



Lane	Description	Lane	Description
1	P17-GOI-DET-STREP	5	ZZ-GOI-DET-STREP
2	CUSF-GOI-DET-STREP	6	SUMO-GOI-DET-STREP
3	FH8-GOI-DET-STREP	7	SNUT-GOI-DET-STREP
4	TRX-GOI-DET-STREP	8	GOI-DET-STREP

Figure 6: Example of an agarose gel illustrating the DNA bands generated from PCR products of the GOI CALM1 (UniProt ID P0DP23) expanded with different solubility tags.

Lane	Description	Lane	Description
1	P17-GOI-DET-STREP	5	ZZ-GOI-DET-STREP
2	CUSF-GOI-DET-STREP	6	SUMO-GOI-DET-STREP
3	FH8-GOI-DET-STREP	7	SNUT-GOI-DET-STREP
4	TRX-GOI-DET-STREP	8	GOI-DET-STREP

Figure 7. Example of a capillary electrophoresis generated electropherogram illustrating the DNA bands generated from PCR products of the GOI rpsA (UniProt ID P0AG67) expanded with different solubility tags

If the purity of the eGene constructs is low with the presence of one or more extra band(s) on the gel, there is a risk to obtain the protein of interest with a significant amount of impurities.

Troubleshooting: For eGene constructs containing more than one band, a band-stab and reamplification is required using the Re-amp Primer Mix provided in the eGene Prep kit. The band-stab and reamplification protocols can be found in the Troubleshooting section of this document.

Storage of the eGene constructs

Purified and normalized eGene constructs can be stored at -20°C short term (days) or -80°C for long term storage.

eGene Optimization / Troubleshooting

1. Band-stab protocol

Note: Always wear UV safety glasses when working with a transilluminator.

Overview: Band-stab is suggested to obtain pure eGene constructs directly from the eGene PCR assembly using the eGene Prep Kit. An example of a band-stabbed gel is in Figure 8.

It is a simple method to obtain single bands as compared to gel purification techniques which requires excision and purification of the DNA fragment from the agarose gel.

The method described in this section is adapted from the publication by Bjourson and Cooper (*Bjourson AJ, Cooper JE. Band-stab PCR: a simple technique for the purification of individual PCR products. Nucleic Acids Res. 1992 Sep 11; 20(17): 4675*).

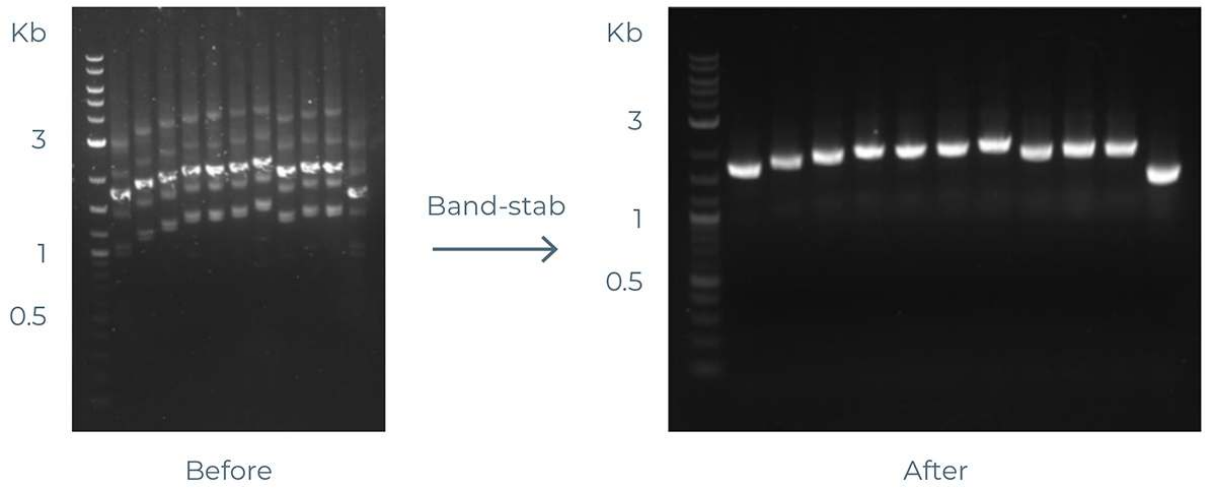


Figure 8: Example of band-stabbed gel.

1. Run the eGene constructs showing non-specific bands on a 1 % (w/v) agarose gel, or use the gel made for the final gel QC step.
2. Load 2 µL of purified product on the gel.
3. Set-up the electrophoresis apparatus for 45 minutes at 120 Volts, or until the desired band is well separated.
4. While the electrophoresis is running, prepare the reamplification PCR reaction as described in Section 2. Reamplification.
5. When the electrophoresis run is complete, take the gel from the tray and take a picture using an imaging apparatus and locate where your desired bands are.
6. Place the gel on the transilluminator and use the blue light setting.
7. Locate your band of interest and take a P10 pipette with a P10 filter tip attached.
8. Hold the pipette vertically on top of the desired band and stab the band three times per PCR reaction.
9. Transfer the pipette tip into the PCR reamplification reaction mix. It is recommended to pipette up and down three times to release all the material from the pipette tip into the PCR reaction solution.

Note: avoid transferring pieces of gel into the PCR reaction mix as they may inhibit the amplification process

2. Reamplification

General information

The reamplification employs the same principles of a regular PCR reaction. This method allows synthesizing more of a eGene construct, either collected from a band-stab protocol, or in the case the quantity is not sufficient for a use on the eProtein Discovery instrument or for a scale-up

experiment.

Notes:
▹ Always use an original eGene assembly as template for PCR reamplification. It is not recommended to use a reamplified product as a template as this would increase the risk of mutations over the subsequent generations.
▹ The minimum number of reactions that should be prepared is 3 x 50 µL:

- The first reaction is with the template eGene construct
- The second reaction serves as a no template control.
- The third reaction is prepared as a spare reaction. Due to the different viscosities and temperatures of the reagents, the pipetting volumes can be inaccurate leading to shortage of reaction solution.

▹ If re-amplifying more than one construct, it is recommended to prepare a sufficient reaction solution containing all the components except the template that are subsequently added to the respective wells or tubes.

▹ Reamplification product lengths are 40 bp shorter than original eGene constructs due to the use of a nested primer pair to synthesize them.

Preparation of the eGene template DNA to be reamplified

1. Prepare a 10 µL solution of the eGene template normalized to 2 nM and keep on ice. Example: For a 2159 bp gene of interest obtained at 10 ng/µL, which corresponds to 7.5 nM, add 3.6 µL of DNA template to 10 µL of nuclease-free water.

Preparation of the reamplification PCR reaction mix

1. Thaw 2x PCR Mastermix and Re-amp Primer Mix at room temperature and spin down tube for 10 seconds at 1000 g.

2. For each reamplification PCR reaction, add the components listed in Table 7 and keep the tubes or the PCR plate on ice

Note: An additional well or PCR tube should be used as negative control, using nuclease free water instead of the eGene.

Reagent	Reaction volume
Nuclease free water (NFW)	21.5 µL
2X PCR mastermix (high fidelity PCR mastermix, user supplied)	25 µL
Re-amp Primer Mix	2.5 µL
eGene DNA template, or nuclease free water	1 µL
Final Volume	50 µL

Table 7. Preparation of the PCR reaction solutions.

Reamplification PCR reaction

1. Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.
2. Place your samples in a thermal block cycler and perform PCR using the parameters in Table 8.

PCR steps	Temperature	Time	Number of cycles
Pre-Incubation	98	30	1
Denaturation	98°C	10 seconds	19
Annealing	60°C	20 seconds	
Elongation	72°C*	30 seconds per kb**	
Final Elongation	72°C*	2 minutes	1

Table 8. Thermocycler parameters

* Elongation temperature: to be based on manufacturer's recommendation.
** Elongation time: to be based on manufacturer's recommendation but should not be less than 30 seconds per kb of the final eGene construct.

3. To evaluate, purify, quantify and normalize the obtained re-amplified eGene constructs, follow the same procedure as from section 'Evaluation of the amplified DNA by agarose gel electrophoresis'.

Circular eGene™ Preparation Kit

Circular eGene™ Prep Kit

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General Information

The Circular eGene™ Prep Kit NC3015 is desgined to convert plasmids received from approved DNA provider to be compatible with the eProtein Discovery™ system.

When placing an order for the Circular eGene™ (plasmids), complete the ordering form with your DNA sequence of choice and select vectors based on the solubility tag options available. Solubility tag, detector tag and Strep purification tag is encoded within the vector sequences, adding these elements to the N- and C-terminus of your protein-of-interest. The schematic of the plasmid structure can be visualised in Figure 1.

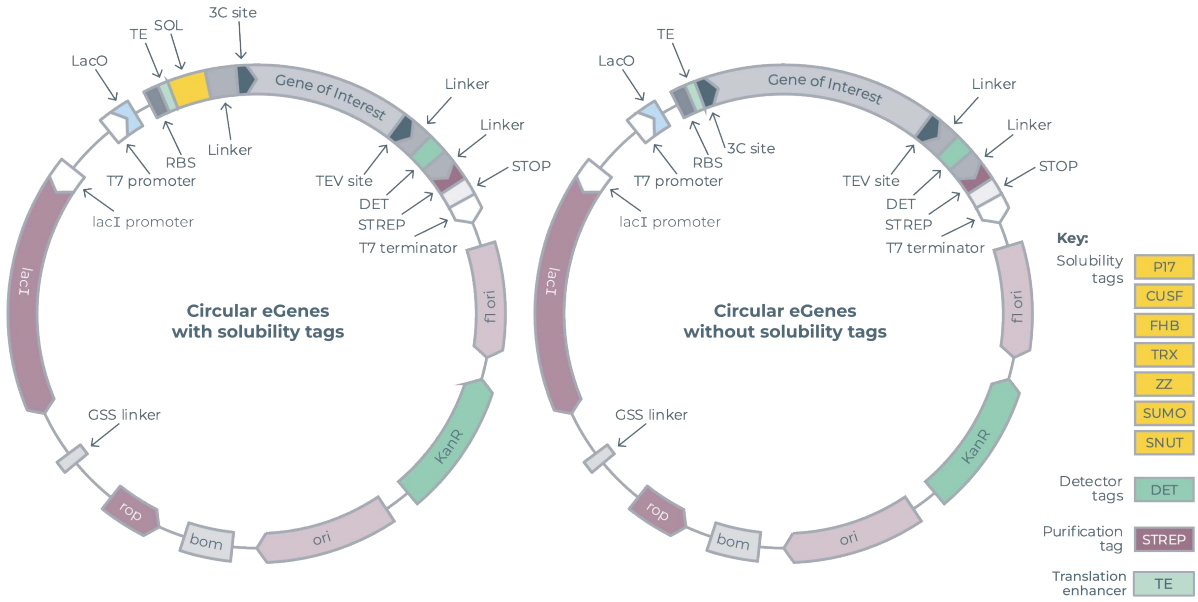

Figure 1: Description of the Circular eGenee™ components. Ribosome binding site (RBS), Translation enhancer (TE), Solubility tag (SOL), 3C and TEV (protease binding sites), Detection tag (DET), streptactin based purification tag (STREP)

Contents

The Circular eGene Prep kit - NC3015 comes with the components listed in Table 1.

The components are supplied in a Nuclera branded box with a green stripe on the label, and must be used within 18 months from the manufacturing date indicated on the kit box.

Component	Volume	Cap Color	Storage Temperature	Tube reference number
Expression	180 µL	Green	-80°C	EBV3-01

Component	Volume	Cap Color	Storage Temperature	Tube reference number
Enahncer				
Expression Enhancer Buffer	350 µL	Yellow	-80°C	EBV3-02
Circular eGene Dilution Reagent	5 mL	White	-80°C	EBV3-03
Nuclease-free Water (NFW)	100 µL	Blue	-80°C	EBV3-04

Table 1. List of components in the eGene Prep kit - Solubility Tag Screen - NC3015.

User supplied reagents / components (not included in the kit)

▹ Circular eGenes at a concentration of 100 nM
▹ Nuclease-free filter tips
▹ 96-well PCR plate or 200 µl PCR strip or Nuclease free 1.5 mL microcentrifuge tubes

User supplied equipment

▹ 37°C incubator or thermocycler
▹ P2, P10, P100 and P200 pipettes

eGene preparation workflow

Figure 2. Circular eGene workflow summary - timings applicable for generating circular eGene constructs using the Circular eGene Prep Kit. For each step of the workflow, the clock indicates the total experiment time and the hand indicates the total hands-on time.

Preparation of circular eGene

> IMPORTANT NOTE
>
> Clean your bench and pipettes with 70% ethanol or isopropanol before starting the work.
> We recommend using nuclease-free fitler tips throughout the protocol to prevent cross-contamination.

▹ Thaw the Expression Enhancer Buffer on ice.
▹ Thaw the Circular eGenes on ice.

Preparation of Enhancer Mix

▹ Label 1.5 mL microcentrifuge tubes as "Enhancer Mix."

▹ Once the Expression Enhancer solutions are completely thawed, briefly spin them to collect all liquid at the bottom of the tube.
▹ Using a 20 µL pipette, add 14 µL of Expression Enhancer, 14 µL nuclease-free water into the pre-labelled microcentrifuge tube. Using a 100 µl pipette add 28 µL of Expression Enhancer Buffer to the same tube.
▹ Set the pipette volume to 50 µL and gently mix by pipetting up and down 12 times. Avoid generating bubbles.
▹ Spin down the Enhancer Mix to collect the liquid at the bottom of the tube.

Preparation of circular-eGene:

▹ Label 24 microcentrifuge tubes or use a PCR plate for each of the 24 circular eGenes. If using a PCR plate, ensure you have a clear and accurate map indicating the position of each gene.
▹ Add 2 µL of the Enhancer Mix to each well/tube using a 2 or 10 µl pipette.
▹ Spin down the plates/tubes at 1000 rpm for 30 sec.
▹ Using a 10 µl pipette, Pipette 8 µL of 100 nM Circular eGene received from your DNA vendor of choice into each labeled tube or PCR plate well (one eGene per tube/well)
▹ Using a 10 µL pipette, gently mix by pipetting up and down 10 times.
▹ If using a PCR plate, seal it with adhesive. Make sure that sealing is good and does not lead to evaporation of the reaction mix.
▹ Spin down the tubes/PCR plate at 1000 rpm for 30 sec.
▹ Incubate at 37 °C for 30 min in a thermocycler or incubator.
▹ If using a thermocycler, set the lid temperature to 47 °C.

Dilution of circular-eGene:

▹ After incubation, remove the tubes/plate from the thermocycler/incubator and briefly spin them.

▹ To each tube or well, add 150 µL of Circular eGene Dilution Reagent.
▹ Gently pipette to mix the solution thoroughly.
▹ Briefly centrifuge the tubes/PCR plate at 1000 rpm for 30 sec.

Transfer and Storage of circular-eGene

▹ Transfer 5 µL of the diluted circular eGene to the Transfer Plate
▹ The remaining sample can be sealed and stored in -80 °C.

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

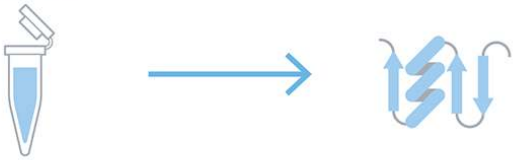
Data driven selection of 30 expression conditions for a strep purifiability assessment to determine purifiable yield

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	

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

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. • 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 • 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 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	• 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

Step	Title	Input	Output	Operations
			Candidate by applying Rule-based Editing - Step 4	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 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

Step	Title	Input	Output	Operations
				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 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

Step	Title	Input	Output	Operations
				structure information (X-ray, NMR, AlphaFold) to guide truncation sites
6	Compile final Screening Candidates	List of input candidates (Step 3) + List of Child Candidates (step 4) + List of Child Candidates (step 5)		• Consider your downstream needs (e.g. 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

Step	Title	Input	Output	Operations
				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.
The decision tree in Figure 2 illustrates the Additive options based on protein requirements.

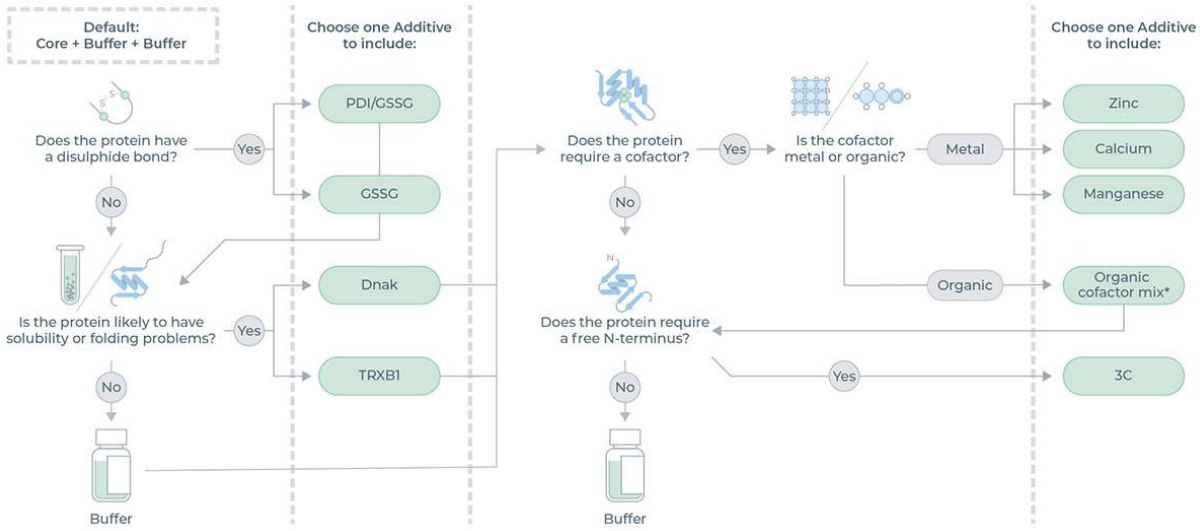


Figure 2: Decision tree which illustrates the Additive options based on protein

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

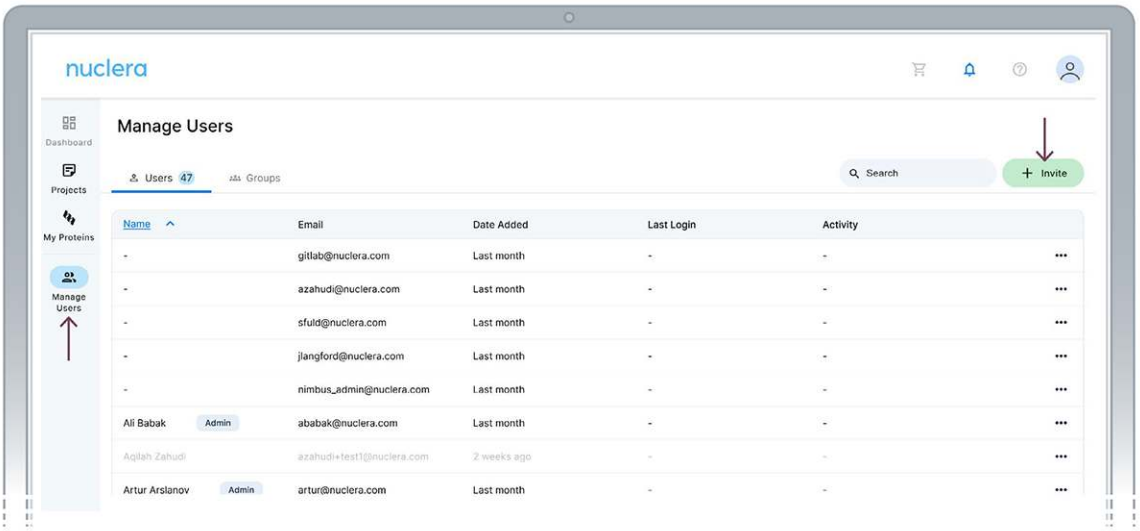
1. The first Administrator user is created by Nuclera.
2. The administrator user will log in to the platform and create projects.
3. The administrator user can invite other users and assign them to projects.
4. Non-administrator users will add their details, create password and an instrument access pin 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

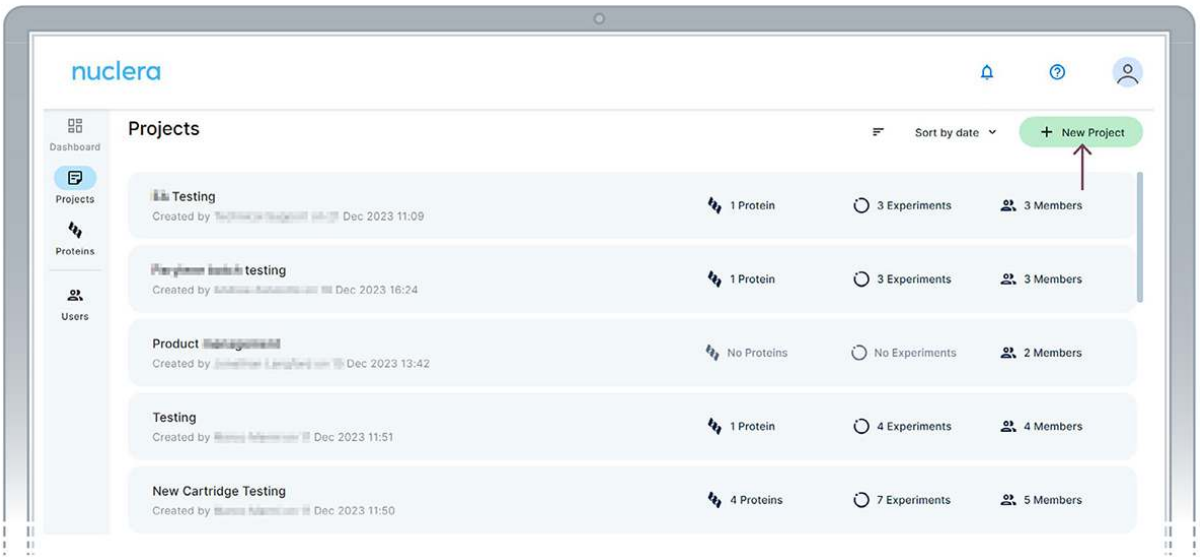
1. From the Manage Users page click the **[Invite]** button.
2. Fill the email address of the invitee and click **[Send]**.
3. The invitee will receive an email with instructions.



Set up a project - Administrator

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

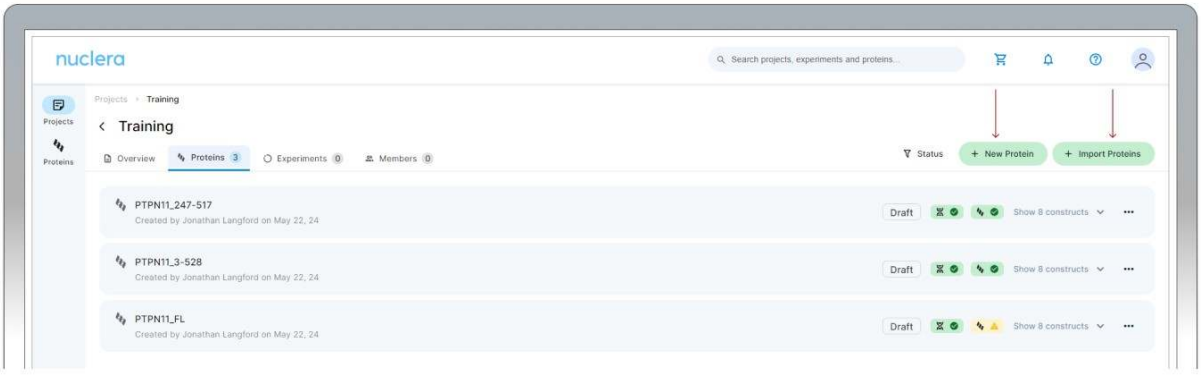
To select an existing project, click on one of the existing projects listed on the screen. To create a new project, click on the **[New Project]** button located on the top right corner of the screen.



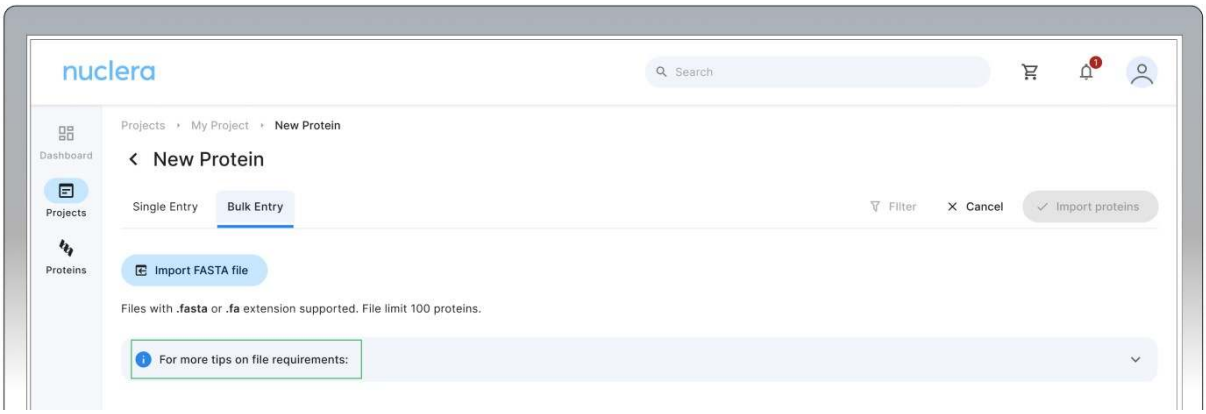
Register a protein sequence - Any user

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

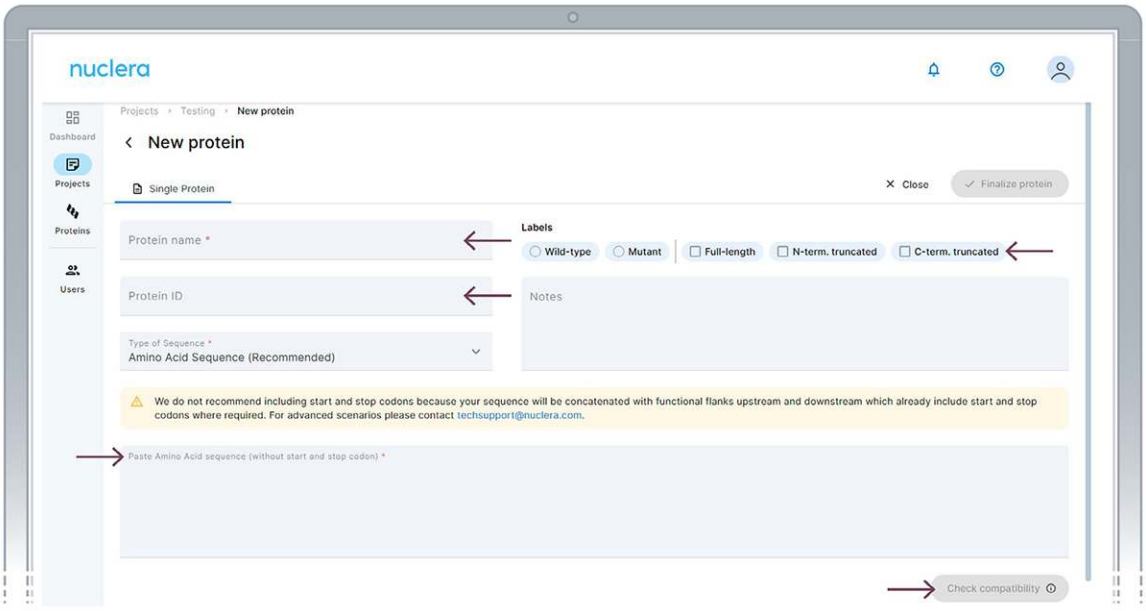
1. Select the **[Proteins]** tab and click the **[+ New Protein]** button.
   Bulk protein sequences can be imported from a FASTA file by clicking on the **[+ Import Proteins]** button. This feature is particularly beneficial for users looking to import 24 sequences or more at once.



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

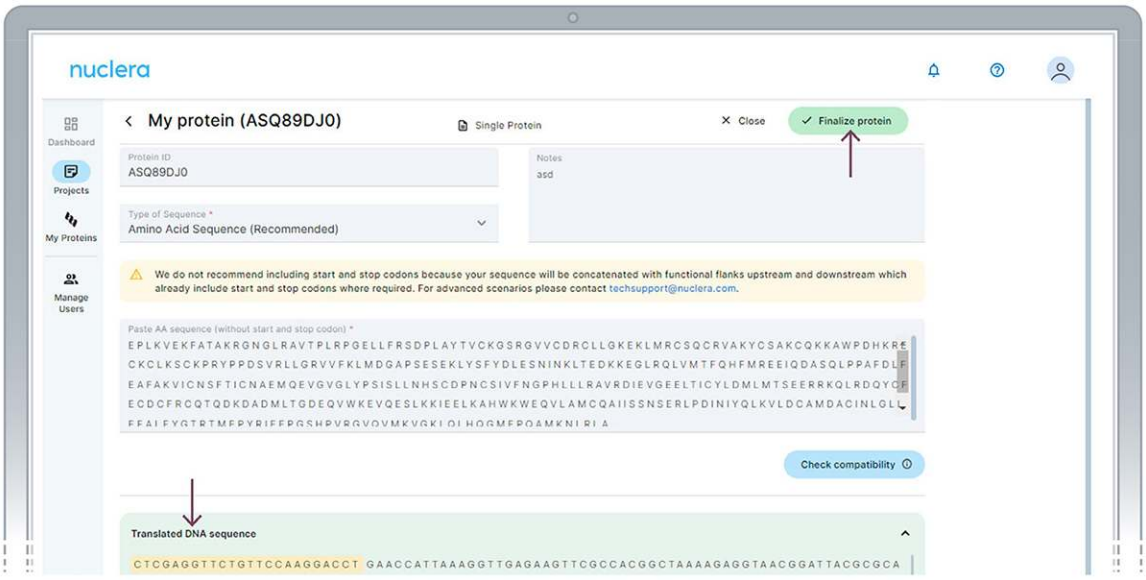


2. Add a name for your sequence and a reference ID (optional). Select the labels that apply to your protein and add any notes. Select the type of sequence you are submitting, amino acid or DNA. Copy and paste the amino acid or a DNA sequence into the input box. Click on **[Check Compatibility]** and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

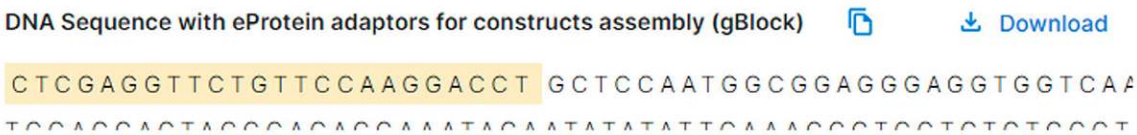


3. If you have uploaded an amino acid sequence, the sequence will be codon optimized and converted to a compatible DNA sequence. Adaptor sequences will also be added to the 5’ and 3’ end. These adaptor sequences will serve as primer annealing points during the PCR reaction to expand each construct with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



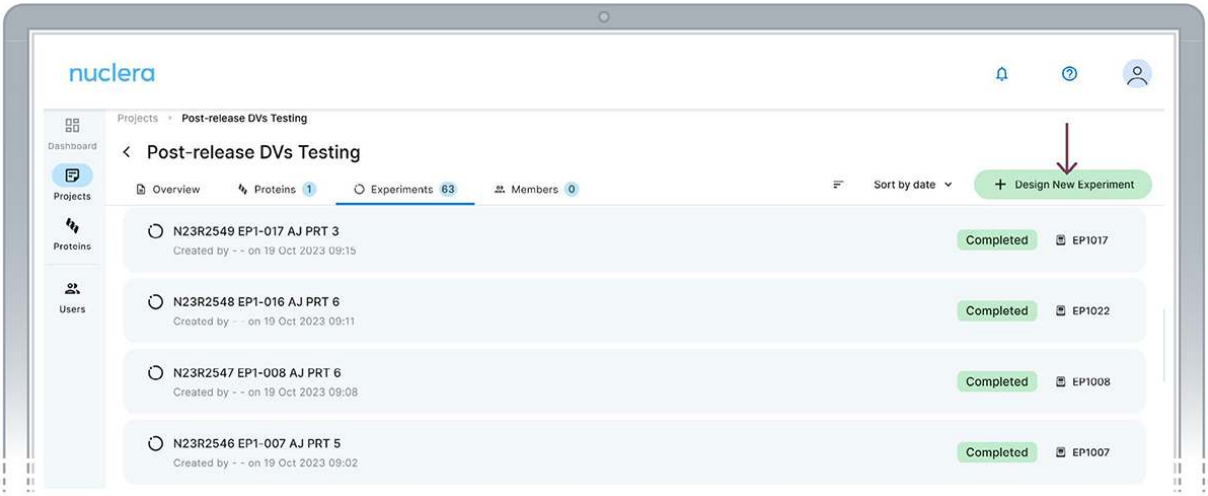
7. Once finalized, the protein or DNA sequence cannot be modified.
8. The translated DNA sequence can be copied and gene fragment ordered from Nuclera's recommended DNA synthesis vendor.



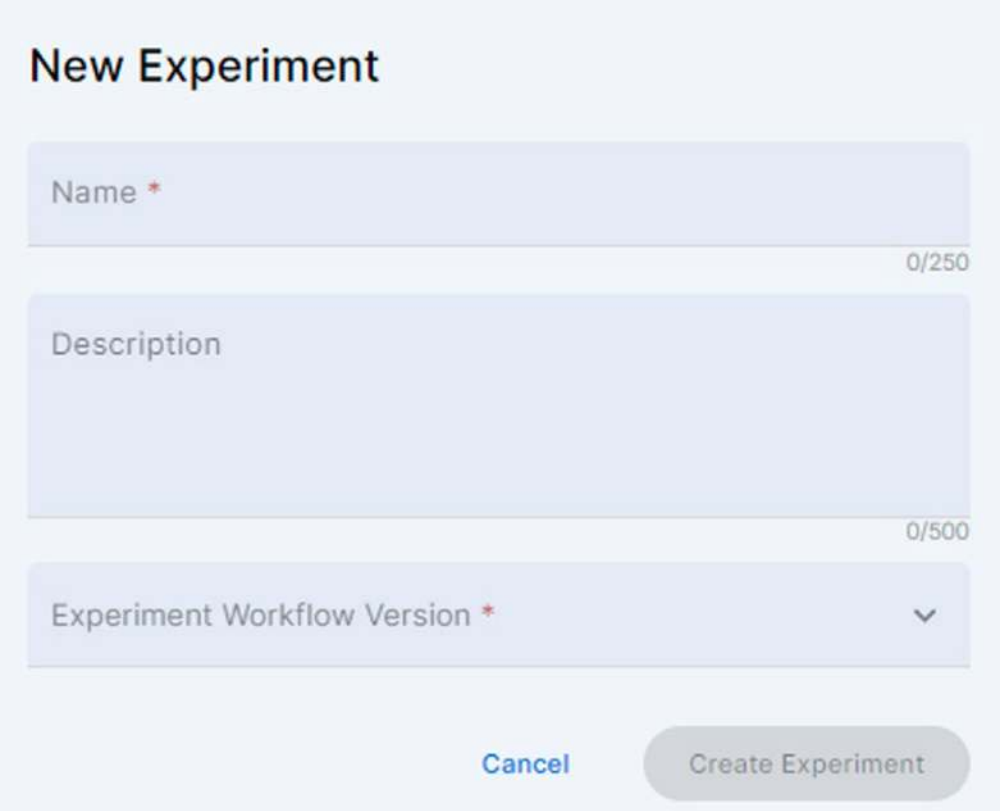
Design an experiment - Any user

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.



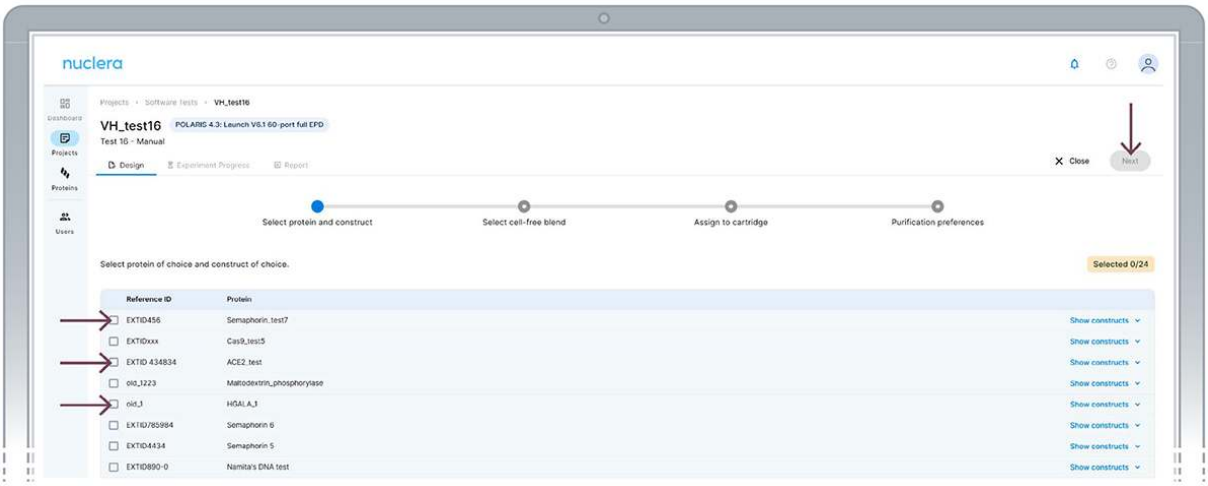
3. Enter the name of the new experiment, add a short text description and select the eProtein Discovery Screen Experiment workflow. Click the activated **[Create Experiment]** button



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."

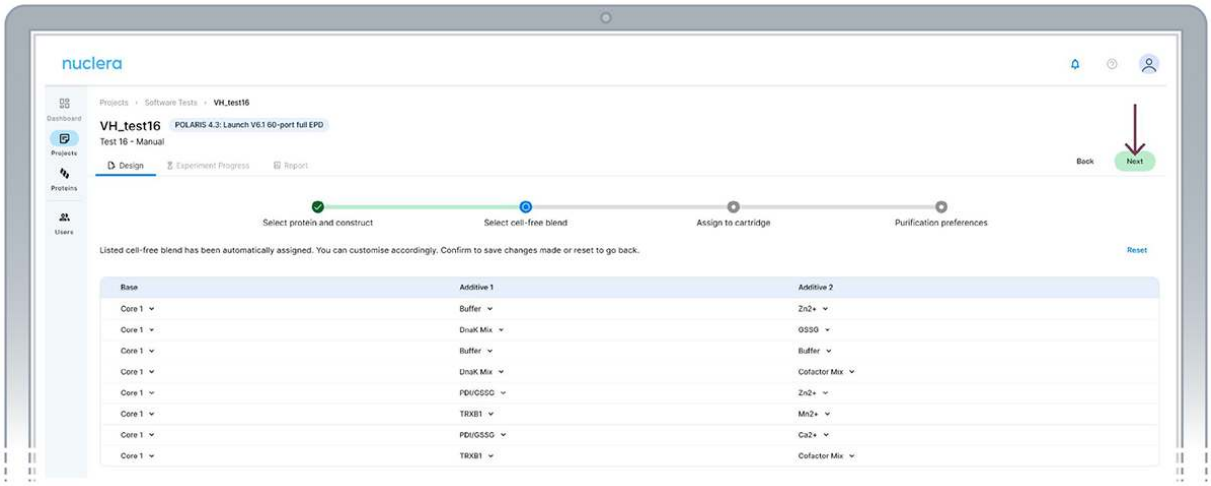
4. Select 24 DNA constructs to assign to a cartridge. Once a desired number of constructs are selected, click on **[Next]**. **Note:** You can load the same construct (duplicate) in two ports if required by your experimental design.



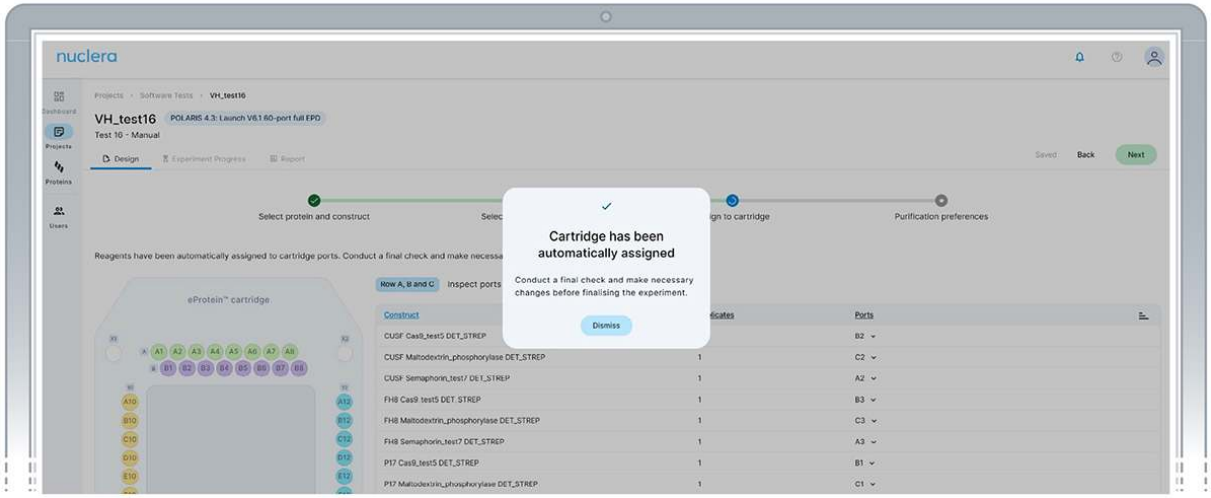
5. Select expression conditions by adding two additives to the Cell-free Core Reagent – The “Additive selection guide” section of this document will help you to select additives. If you are undecided on the additive combinations to use, go with the default list of high performing Cell-free Blends pre-selected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

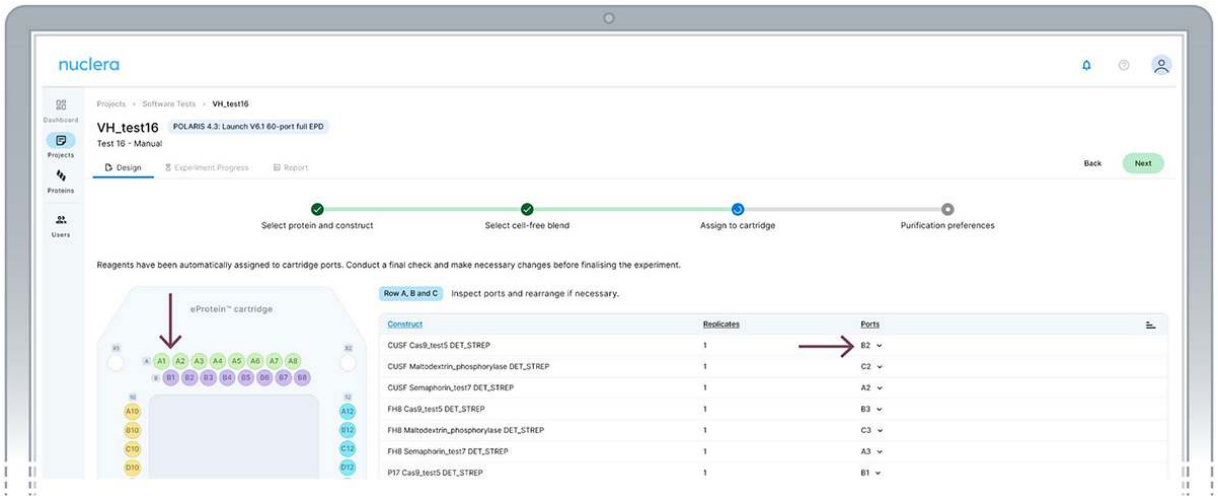
> NOTE
>
> An additive can be selected twice to enhance a specific condition.



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

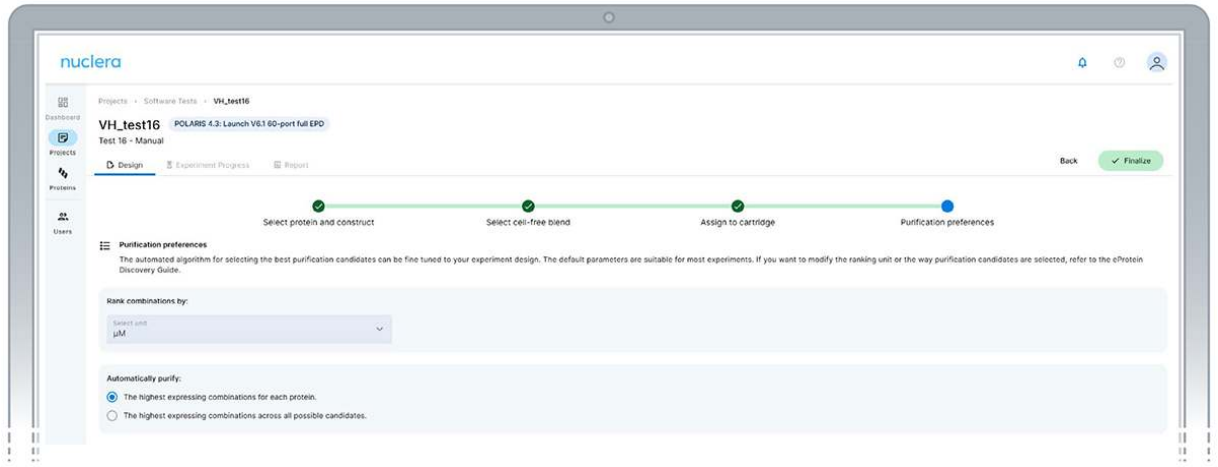


7. Review & Correct sample allocation – In the final step you have the opportunity to inspect and potentially change port location for DNA samples and Cell-free Blends. Drag & drop in the interactive cartridge map or use the port menu available to every sample. Press the **[Next]** button to proceed.



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.

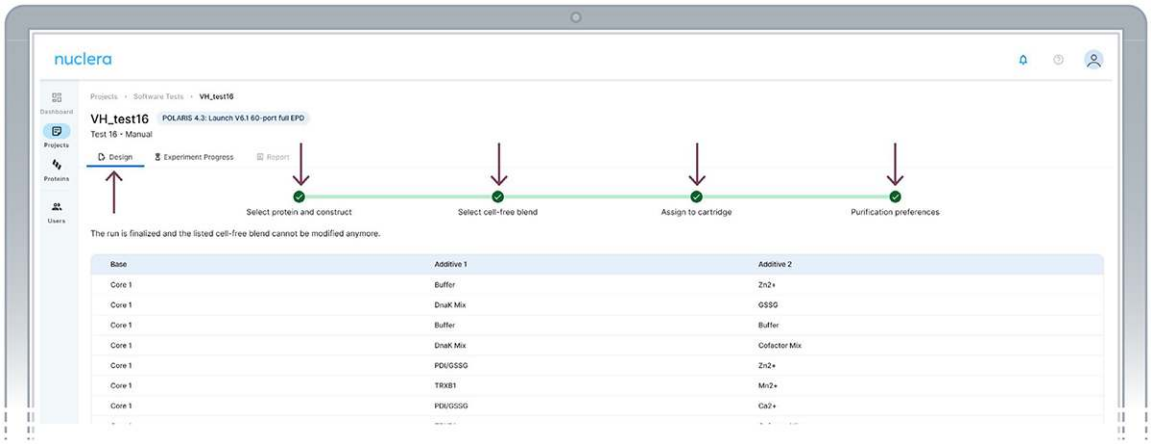
The ranking can be performed using different units, µM or mg/mL.

> 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]**).

Product Image

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).

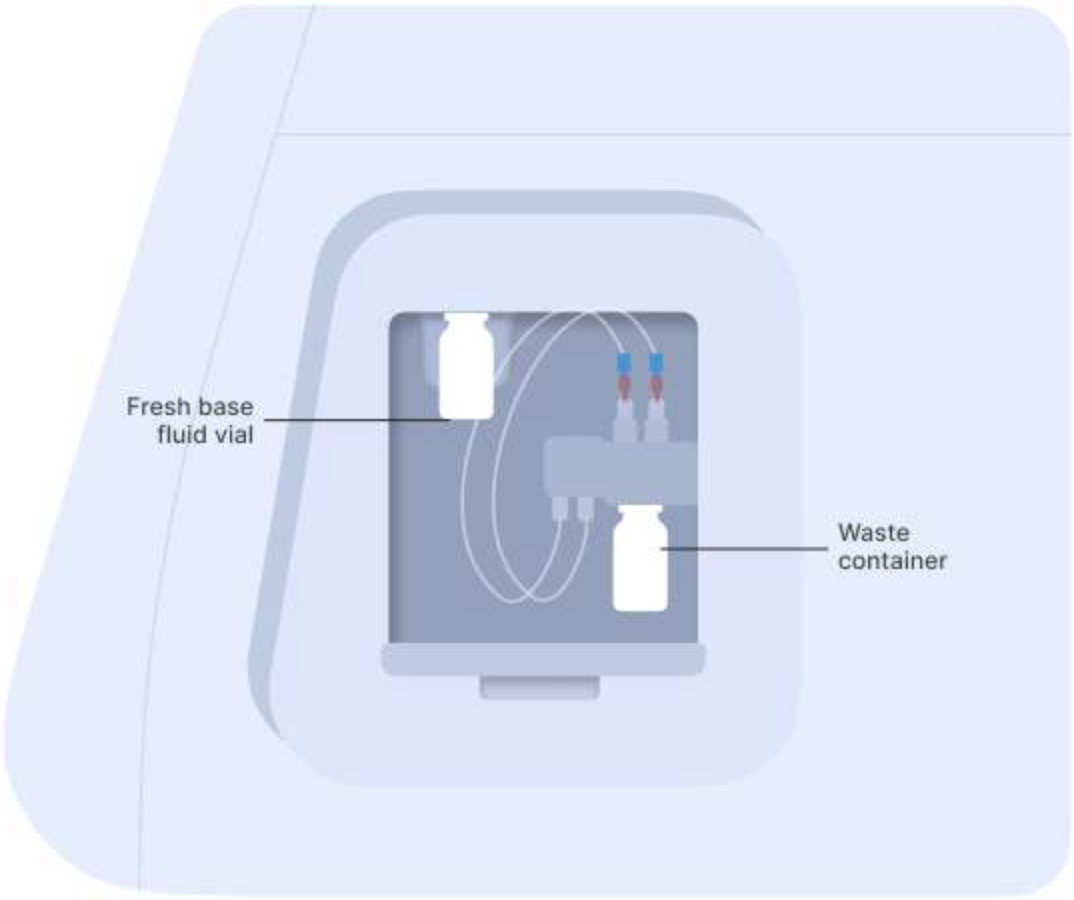


Figure 3: Vial of base fluid and the waste container connected to the pump as shown on the screen

[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.

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.

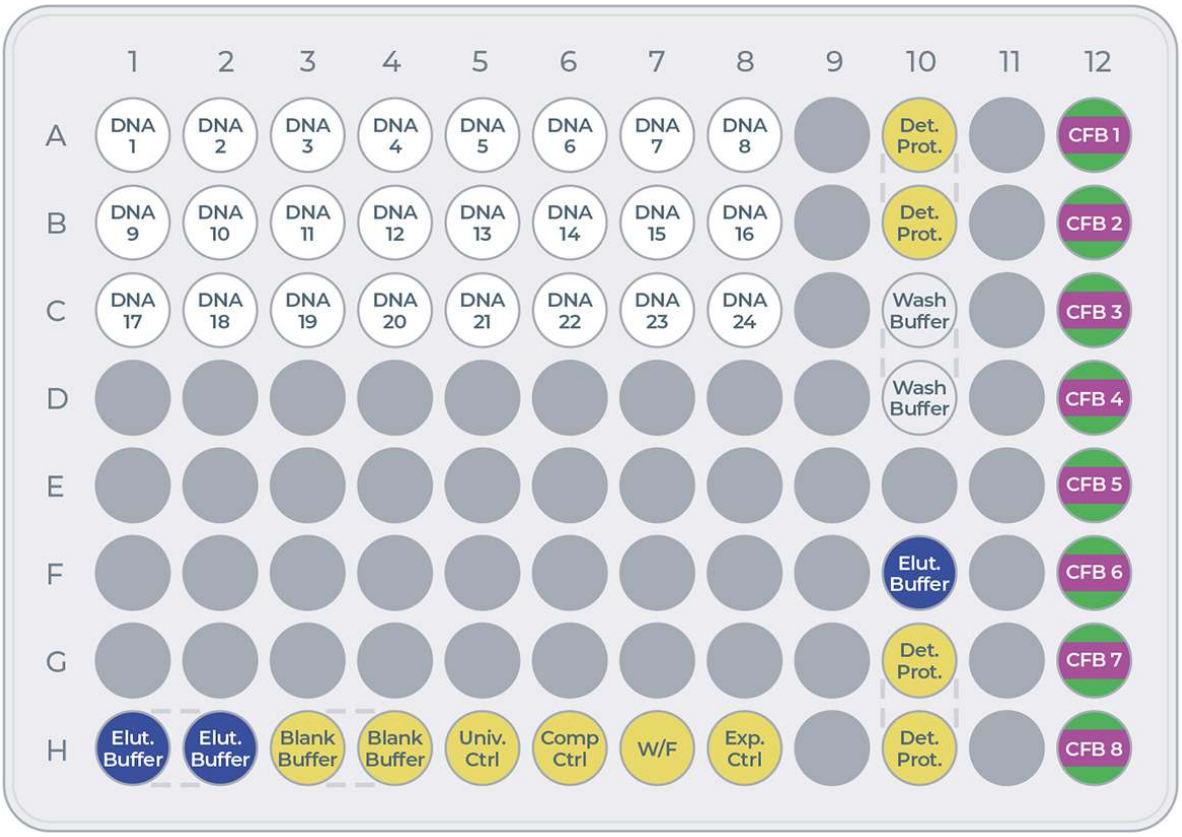


Figure 5: Transfer plate layout

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)

Reagent	Volume (µL)
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).

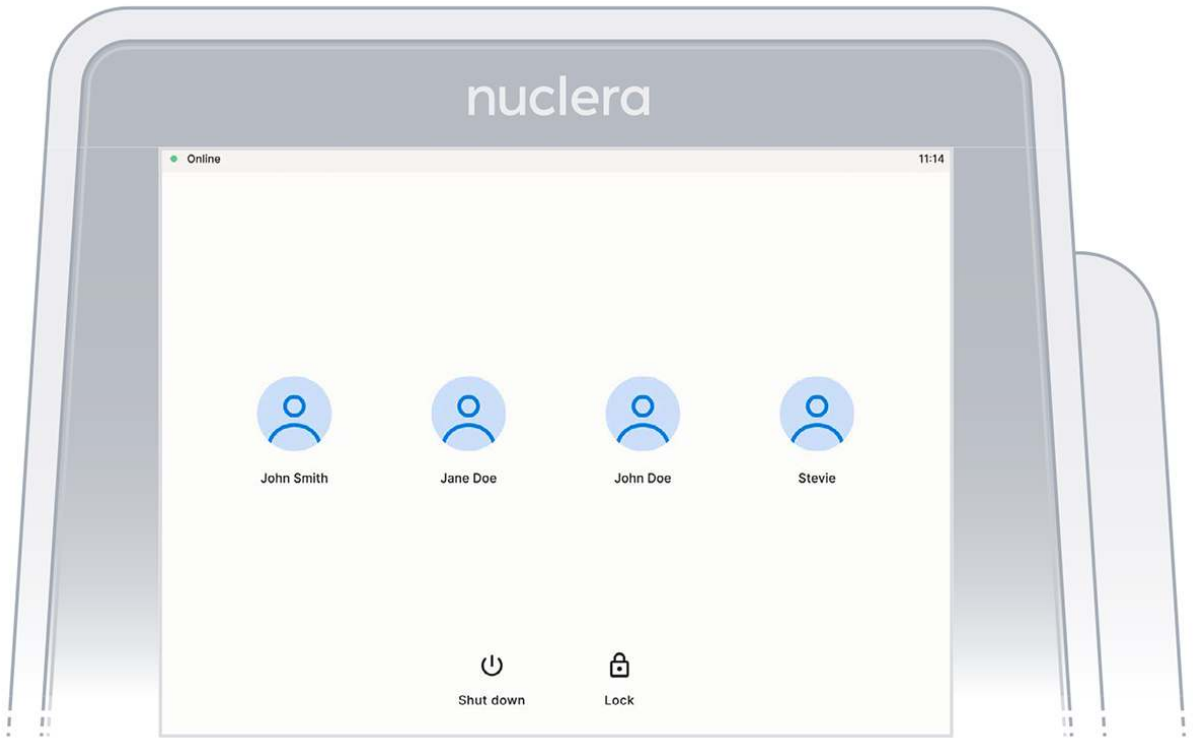


Figure 6a: User accounts on the instrument software

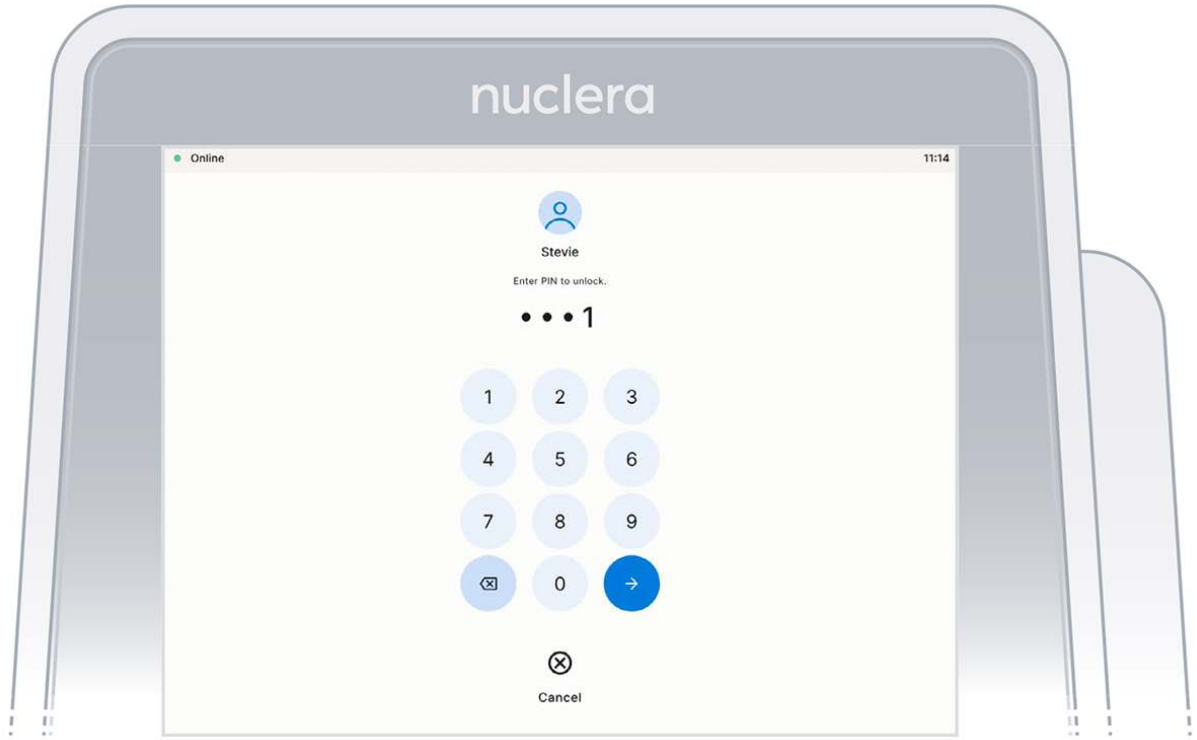


Figure 6b: PIN Interface

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

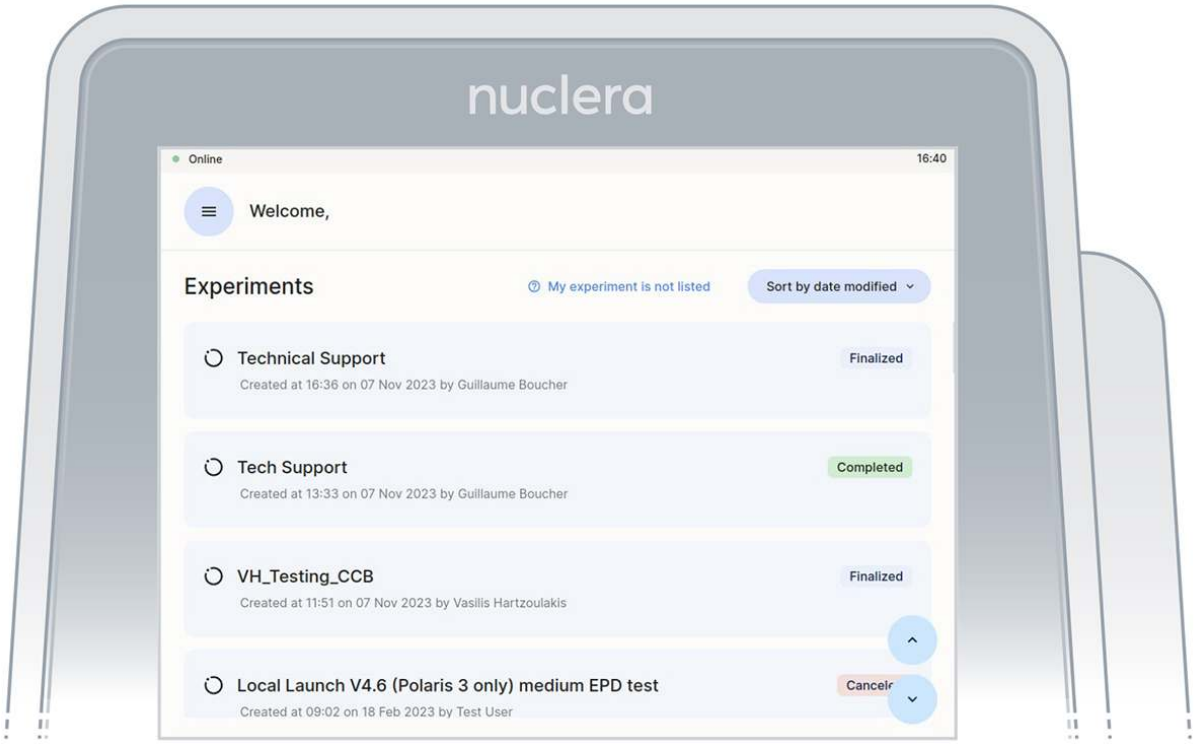


Figure 7: Instrument software welcome page with the list of finalized experiments

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

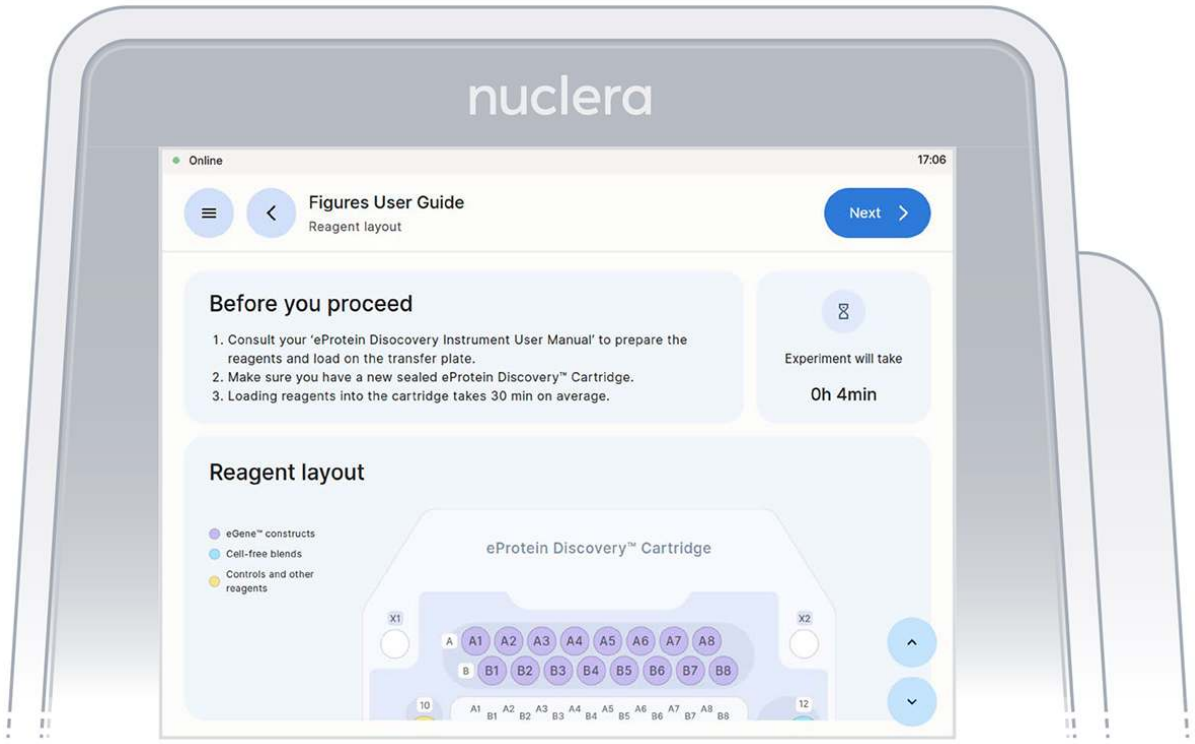


Figure 8: Instrument software welcome page with the list of finalized experiments

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.

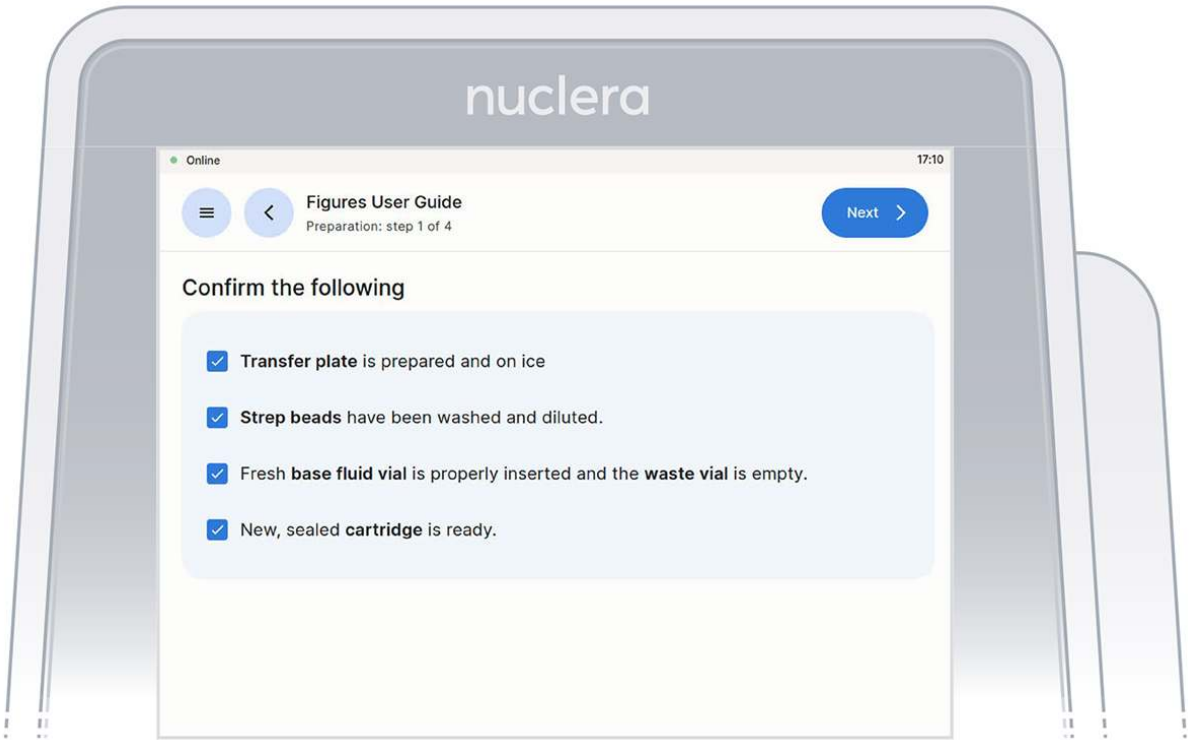


Figure 9: Loading of the cartridge on the eProtein Discovery instrument.

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).

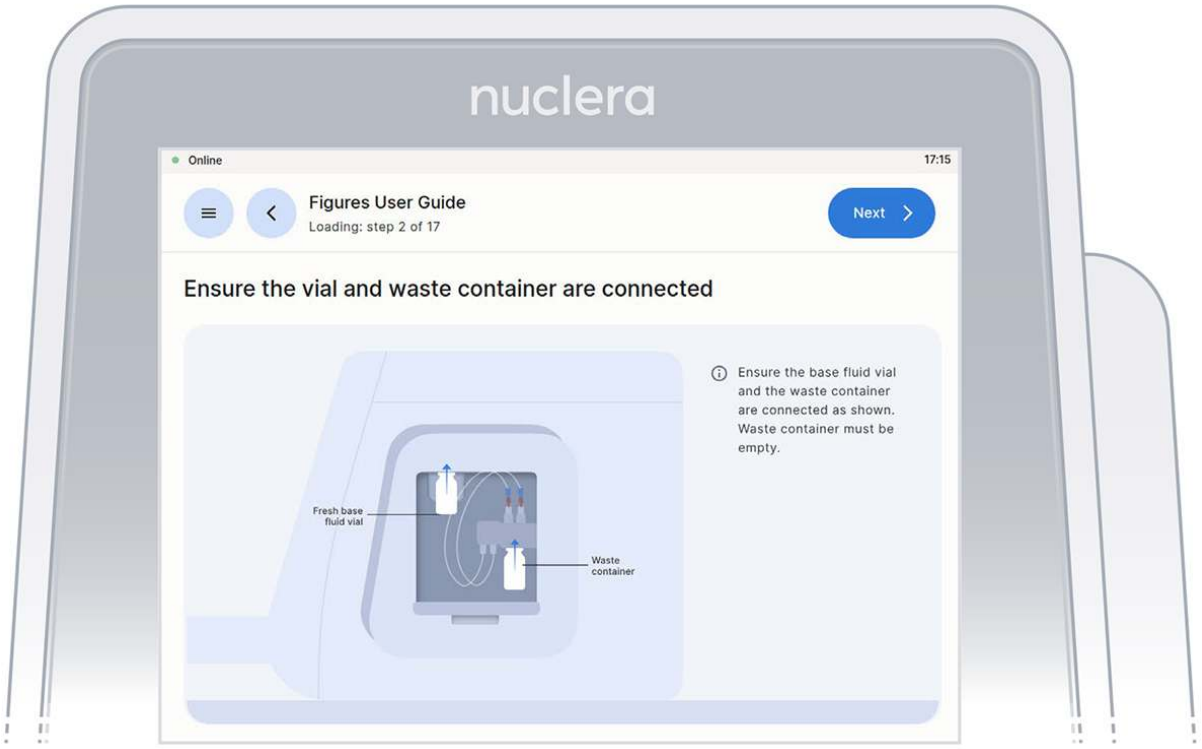


Figure 12: Vial of base fluid and the waste container connected to the pump as shown on the screen

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.

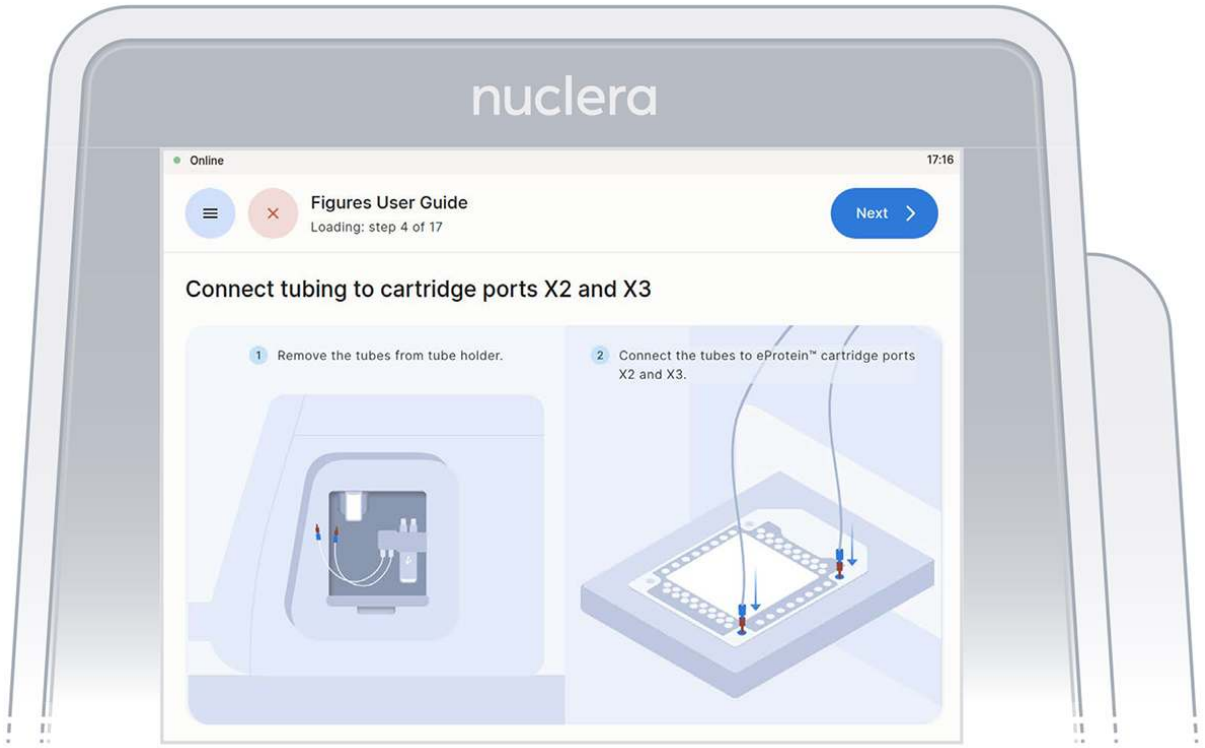


Figure 14: Inspection that all the ports on the cartridge are filled with base fluid

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).

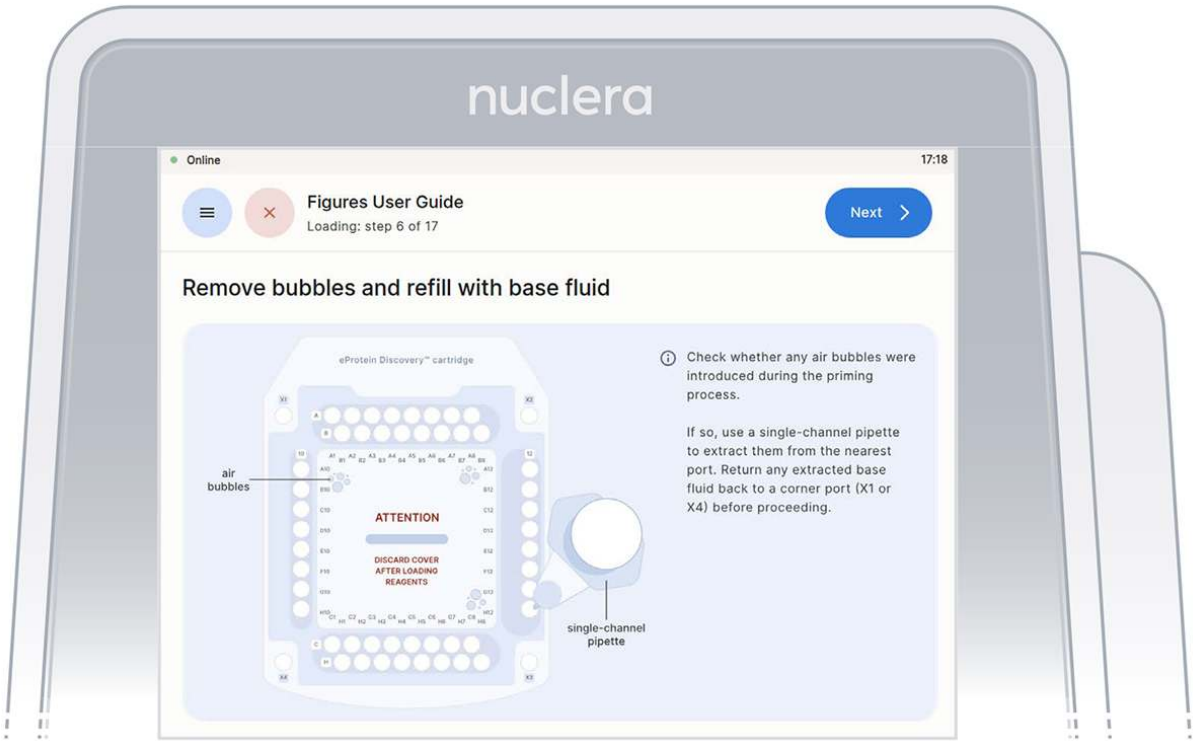


Figure 15: Connection of the pump tubes to the cartridge

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).

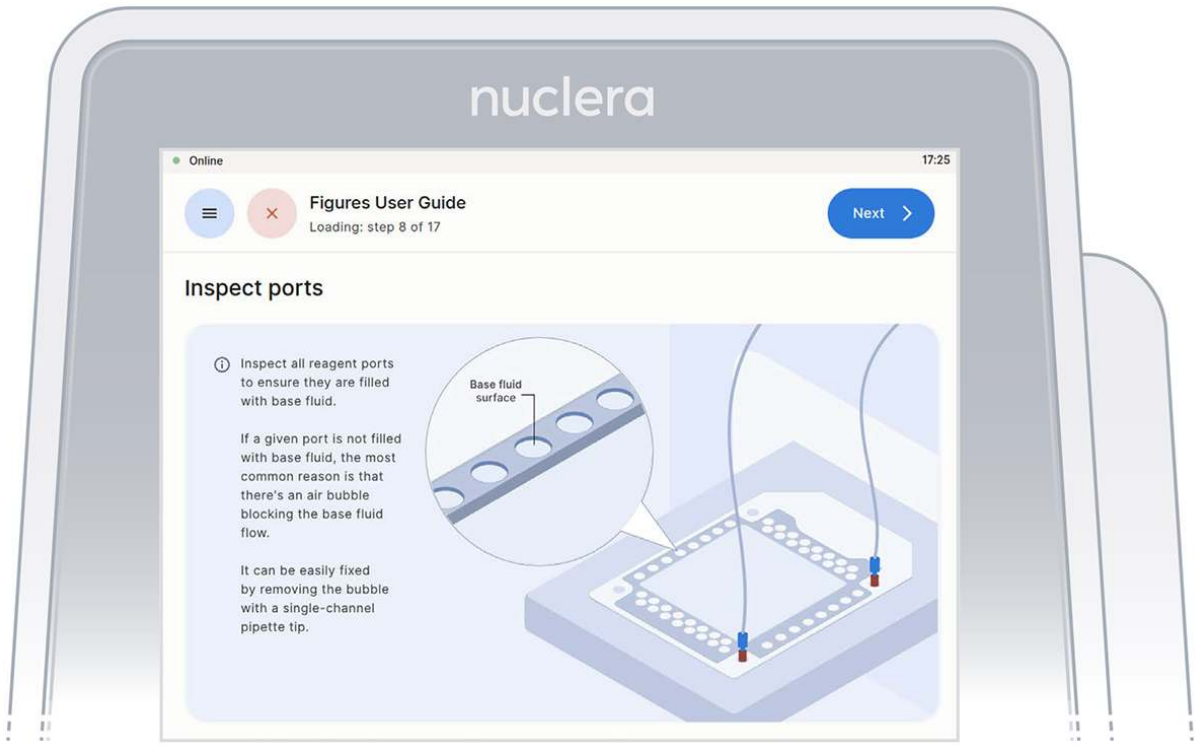


Figure 16:Inspection that all the ports on the cartridge are filled with base fluid

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).

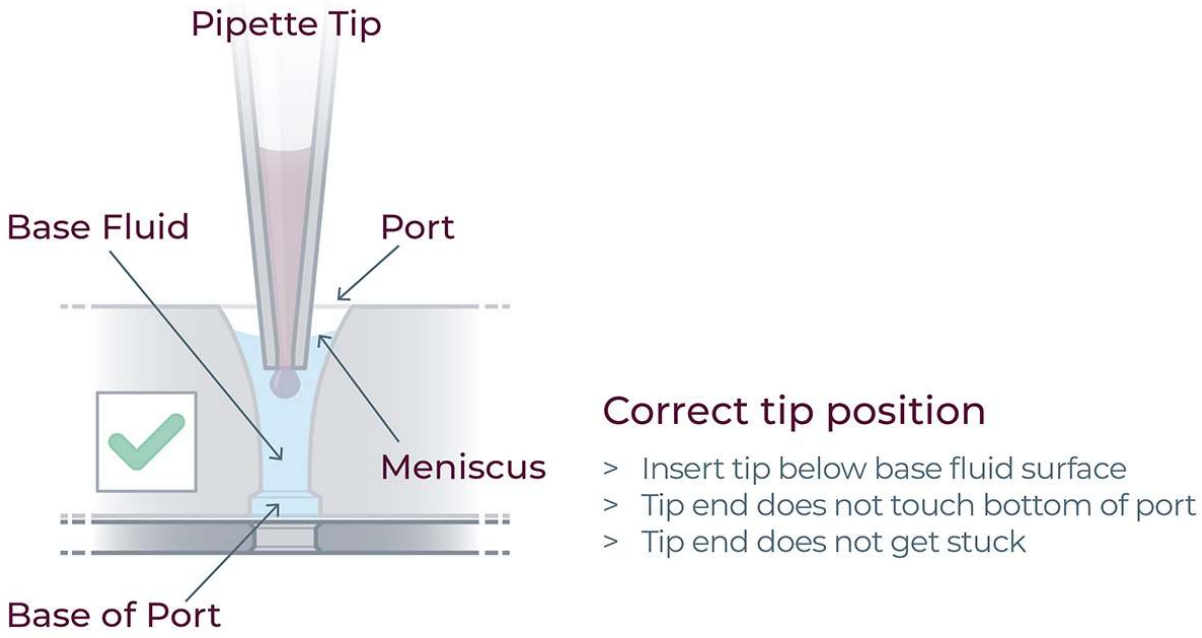


Figure 17: For correct reagent loading the pipette tip is immersed in the base fluid and not touching the bottom of the port

Load eGene constructs - rows A, B and C

▹ 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.

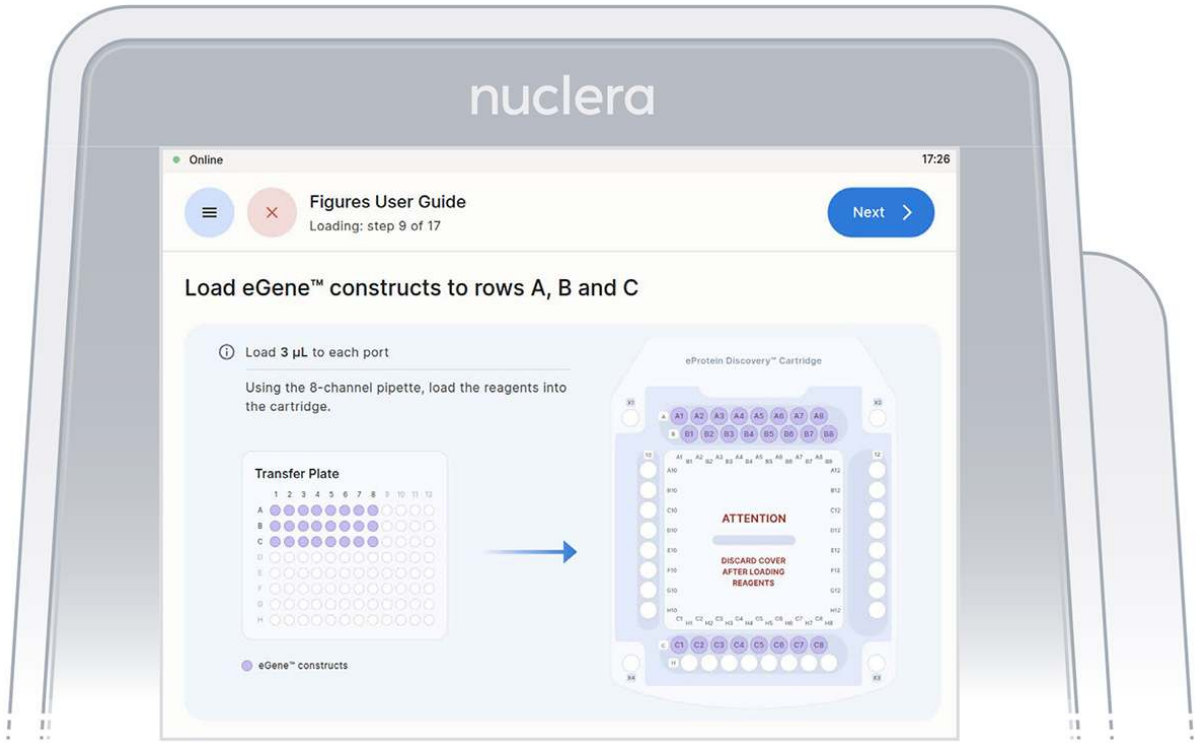


Figure 18: Loading of the eGene constructs onto row A, B and C of the cartridge

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.

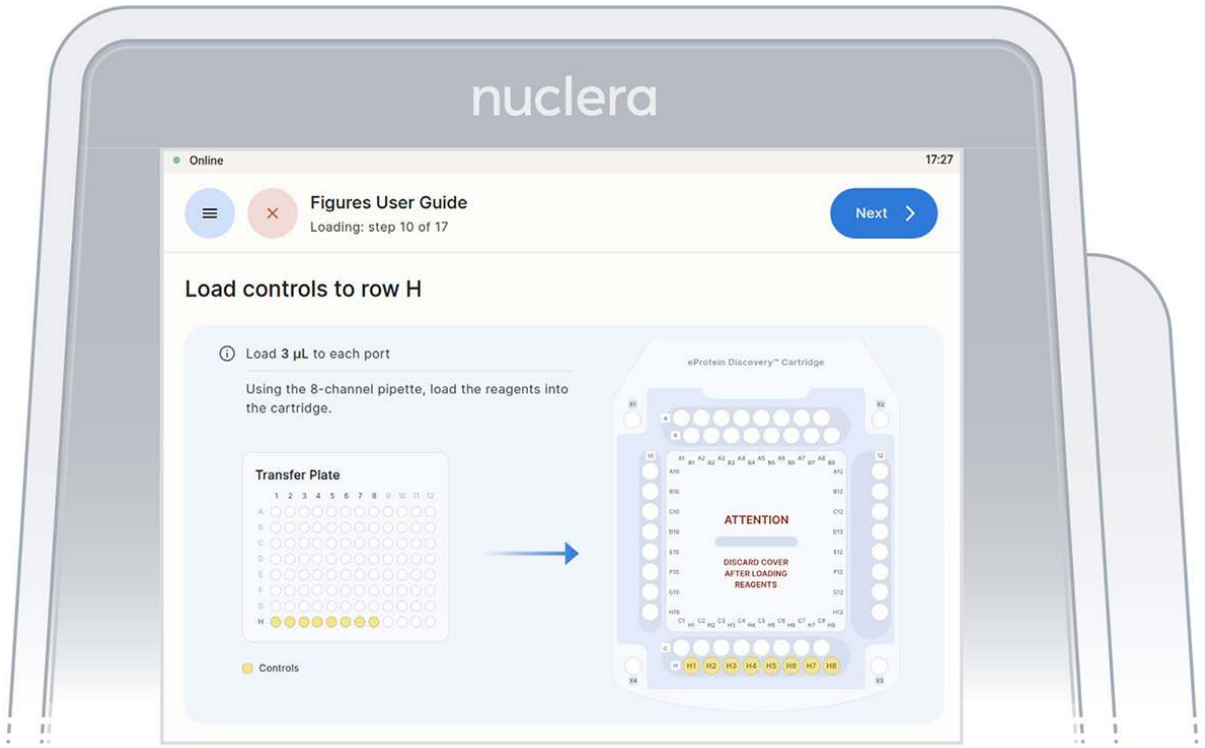


Figure 19: Loading of the reagents onto row H of the cartridge

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.

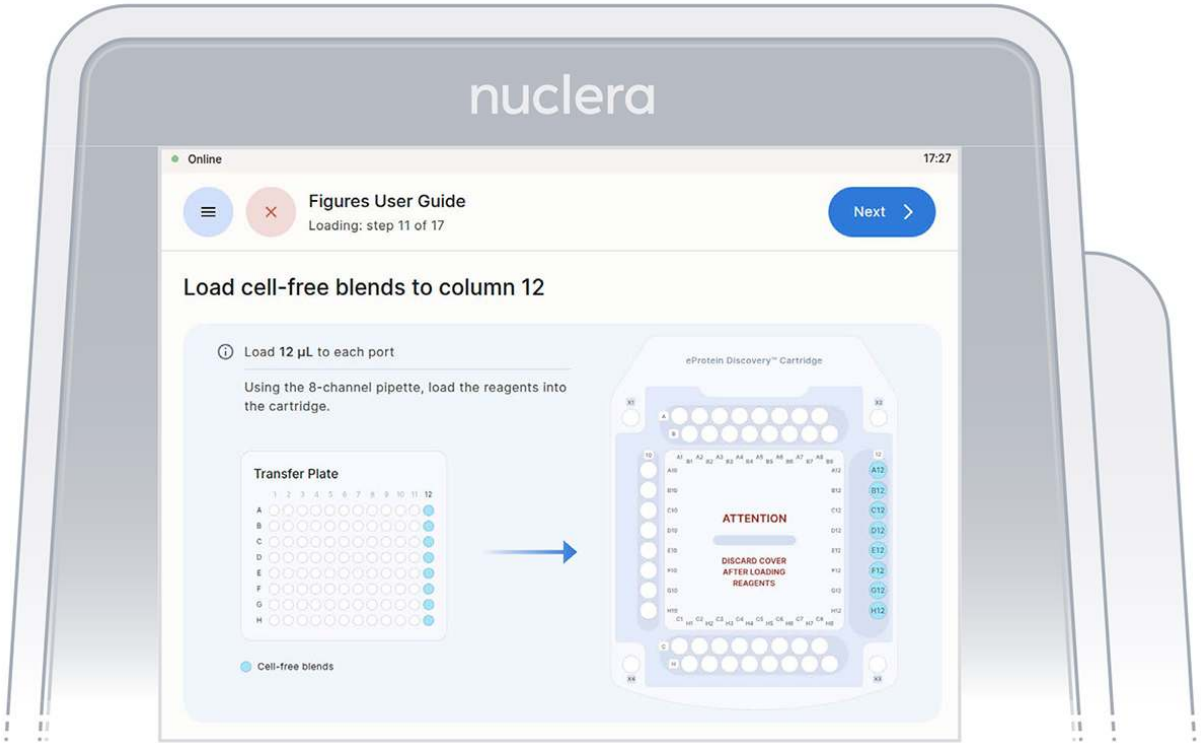


Figure 20: Loading of the Cell-free Blends onto column 12 of the cartridge

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.

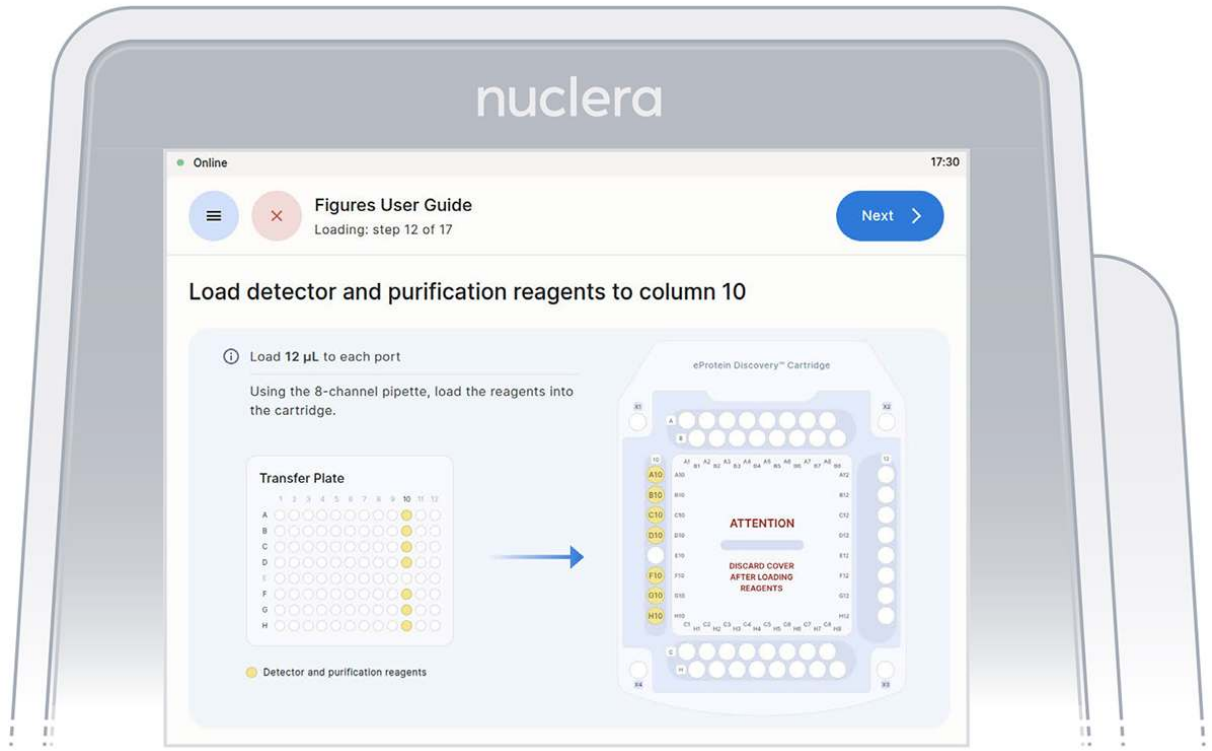


Figure 21: Loading of the reagents onto column 10 of the cartridge

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)

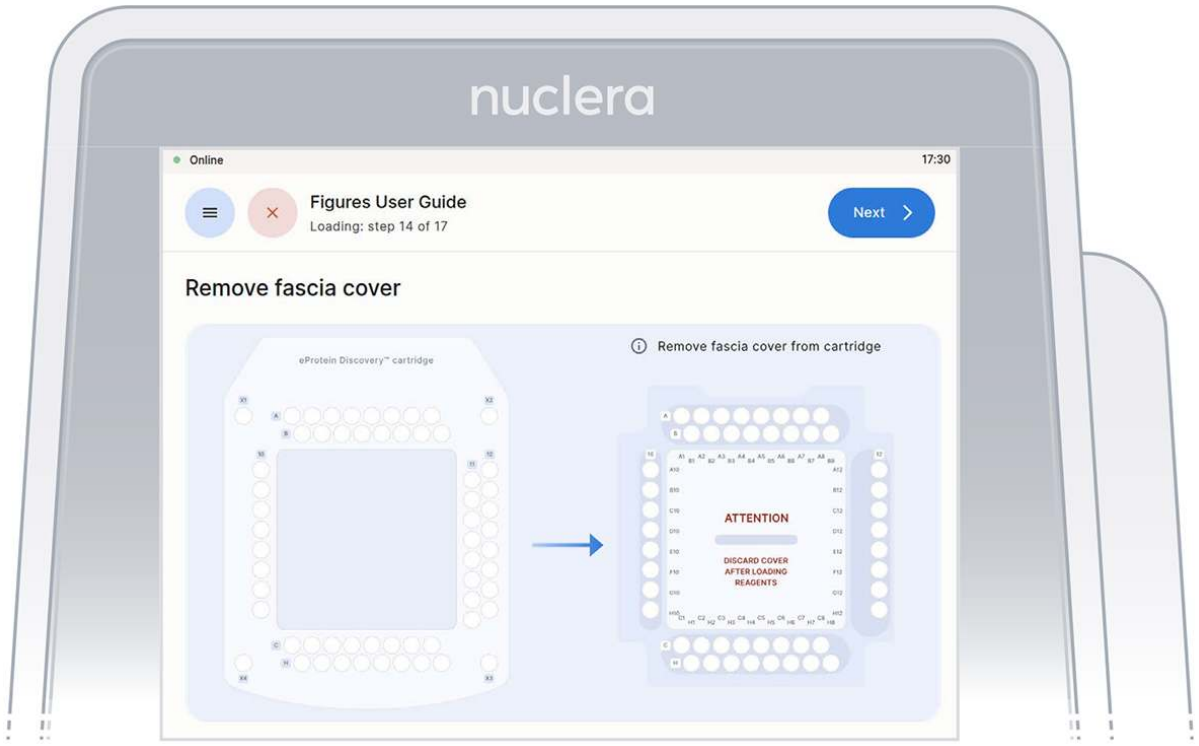


Figure 23: Remove the cover from the cartridge

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).

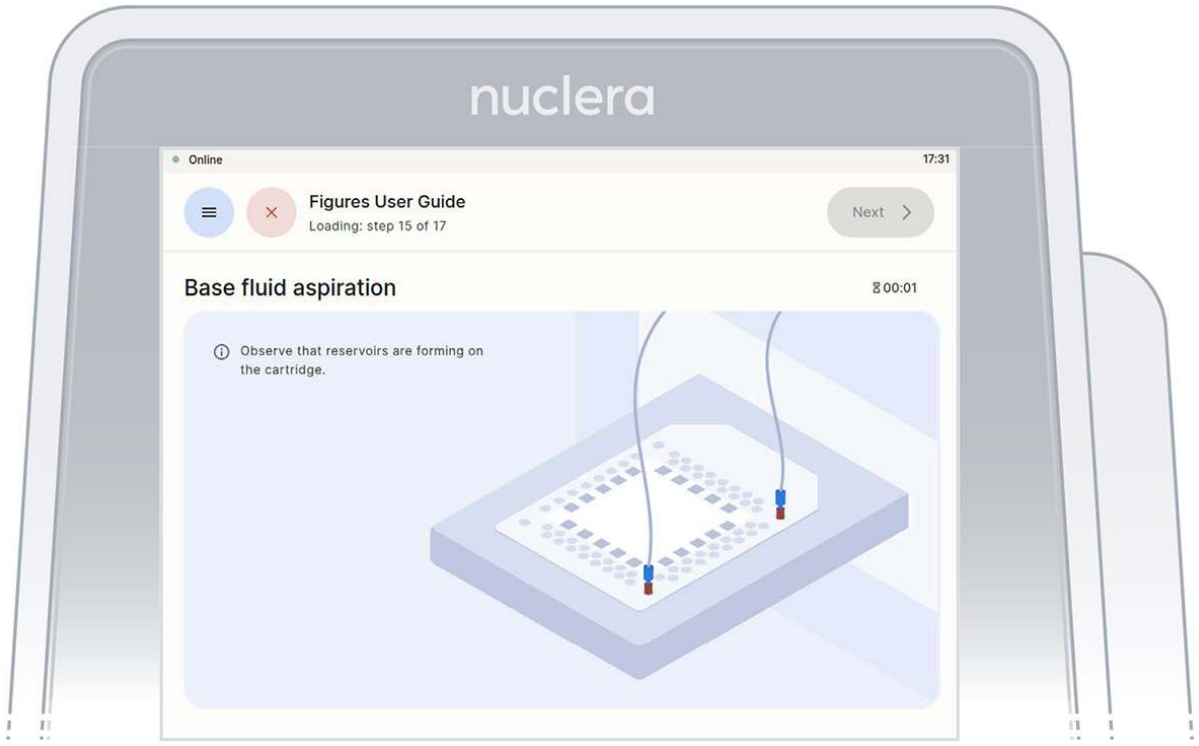


Figure 24: Base fluid aspiration

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.

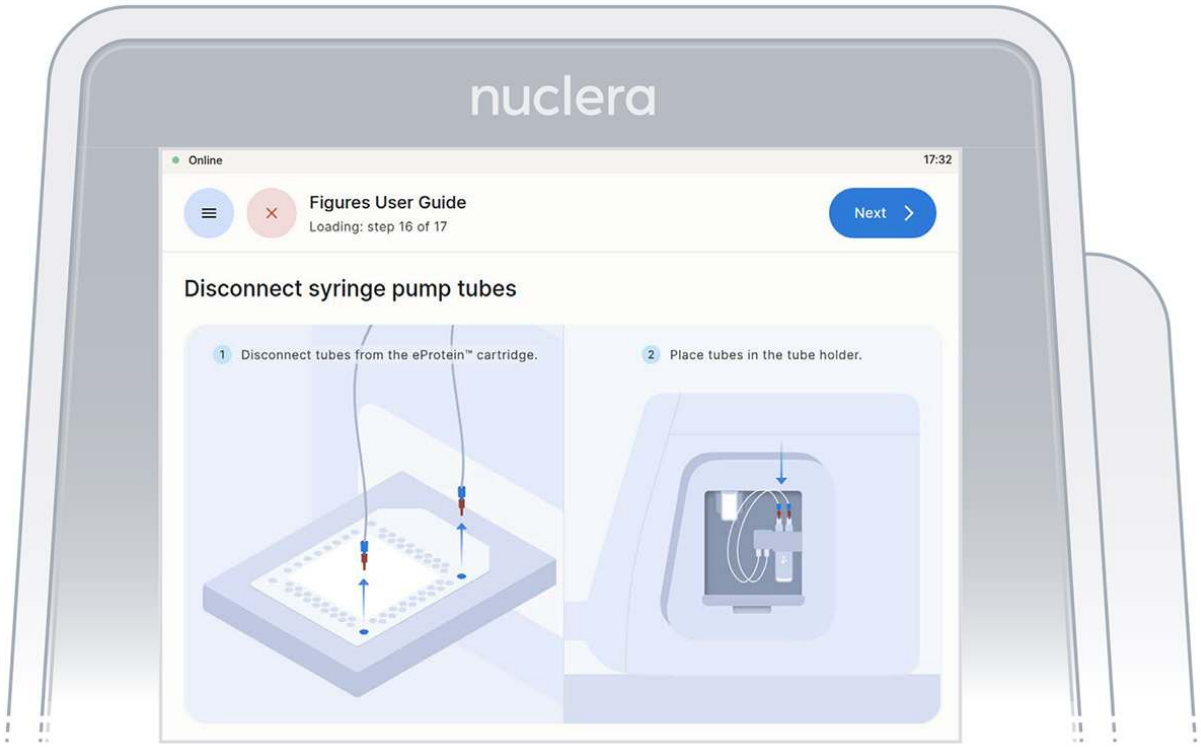


Figure 25: Disconnect the tubes and place them on the tube holder

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

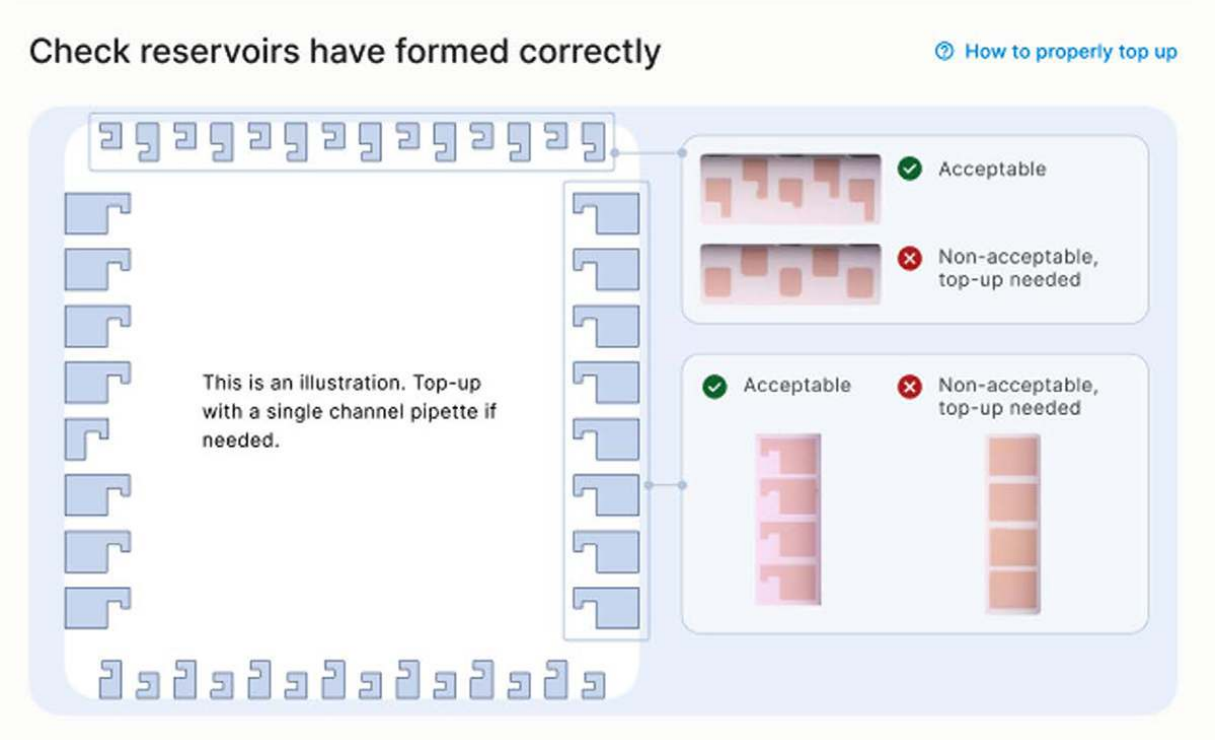


Figure 26: Check the reservoirs have formed correctly on the cartridge

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

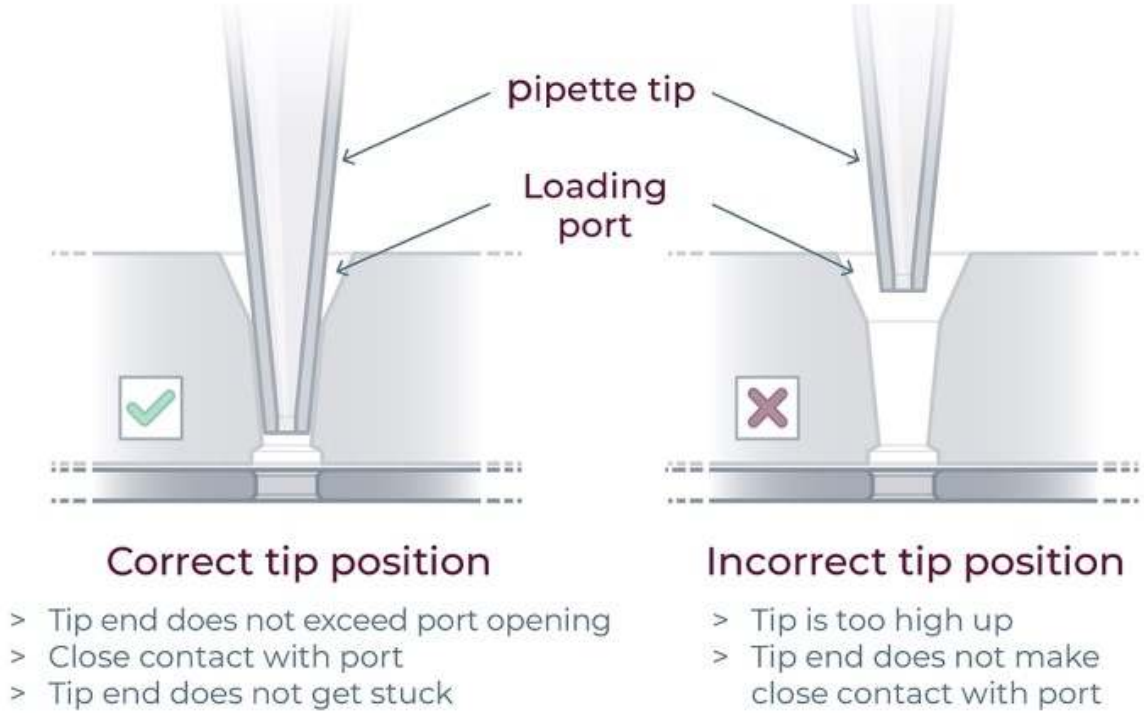


Figure 27: For manual correction of the reagent loading to correctly form the reservoirs on cartridge

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.

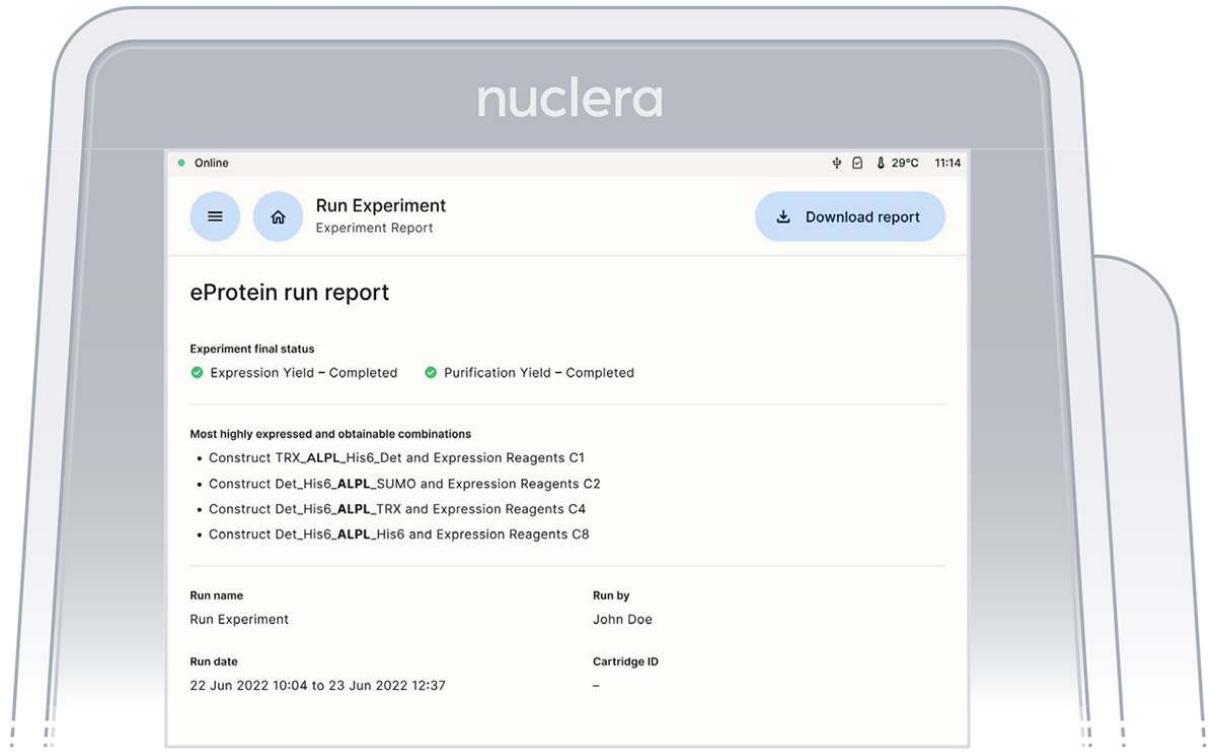


Figure 28: Result screen from the Instrument Software

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)

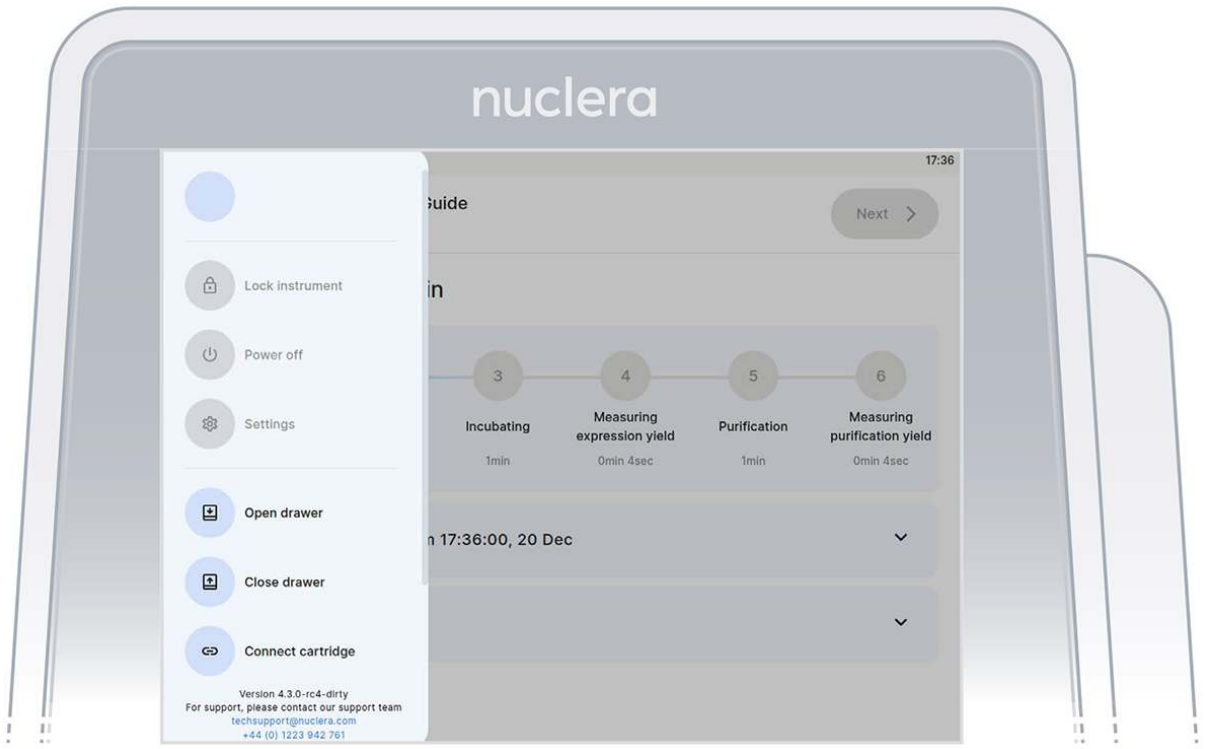


Figure 30: Remove the cartridge from the instrument

Frequently Asked Questions (FAQ)

1. General

Questions/ Issues	Answers
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)
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.

Questions/ Issues	Answers
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 2. Check the socket is working. 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 2. Check that the instrument is connected to the internet through an ethernet cable. 3. If no experiment is running and the data export has completed, restart the instrument.
On the eProtein Discovery software, I	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.

Questions/ Issues	Answers
don’t remember my username / password	
On the Instrument Software, I don’t remember my username / password	Contact your administrator or the Nuclera Technical 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	Contact your administrator or the Nuclera Technical Support team.

Questions/ Issues	Answers
and password are correct but not recognised	
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	If a given port is not filled with base fluid, the most

Questions/ Issues	Answers
port not filled with base fluid	common reason is that there is an air bubble blocking the base fluid flow. This can be
easily fixed by removing the bubble with a single-channel pipette tip	

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

📄 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. 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 96 different combinations in 22 hours for you to select the optimal conditions to scale up and get protein.

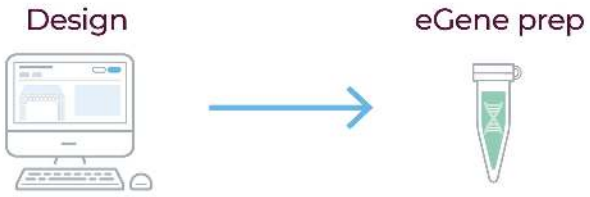
Our eProtein Discovery™ software will guide you in creating the panel of

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

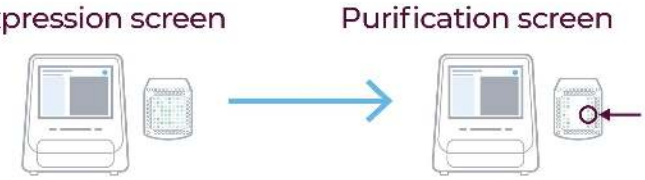


Input protein sequences into software and customize construct (eGene™) design with pre-set fusion tags

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

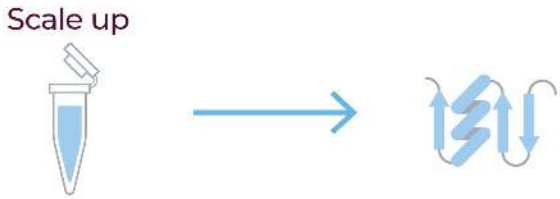
11 eGenes × 8 Customizable Cell-Free Blends = 88 Expression datapoints

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. • 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 • 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. 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
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. • 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 interest. Users may add up to 10 aa upstream or downstream. • Consider truncating to

Step	Title	Input	Output	Operations
				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) + List of Child Candidates (step 4) + List of		• Consider your downstream needs (e.g. activity assay, binding assay, structural investigation) • Consider your cartridge format (e.g. a

Step	Title	Input	Output	Operations
		Child Candidates (step 5)		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

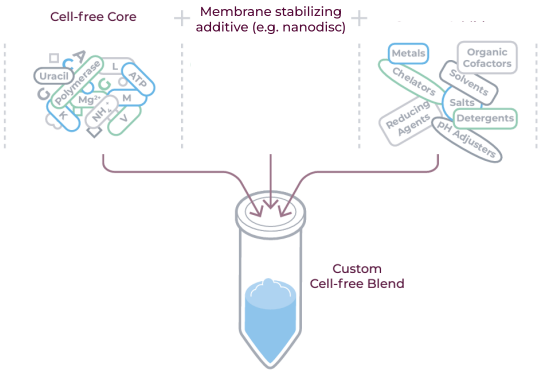
Cell-free expression conditions, termed Cell-free Blends, consist of a Cell-free Core reagent supplemented with two additives tailored towards your protein of interest. Nuclera provides standard additives within the Cartridge Reagent Kit (NC3013), but custom additives can also be introduced, provided 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				✔

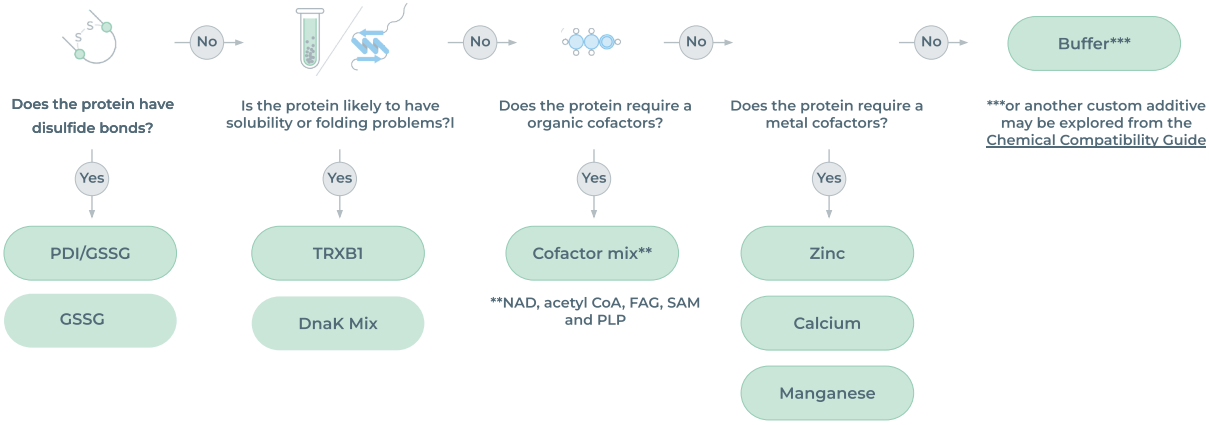
Step 2: Select between optional tags.

- 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

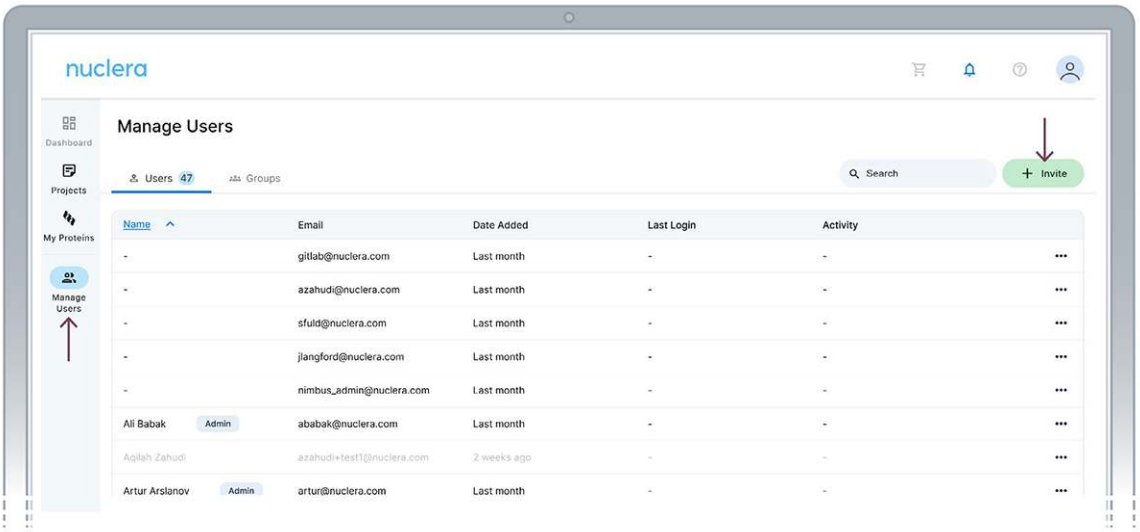
The following steps are required to access our service

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

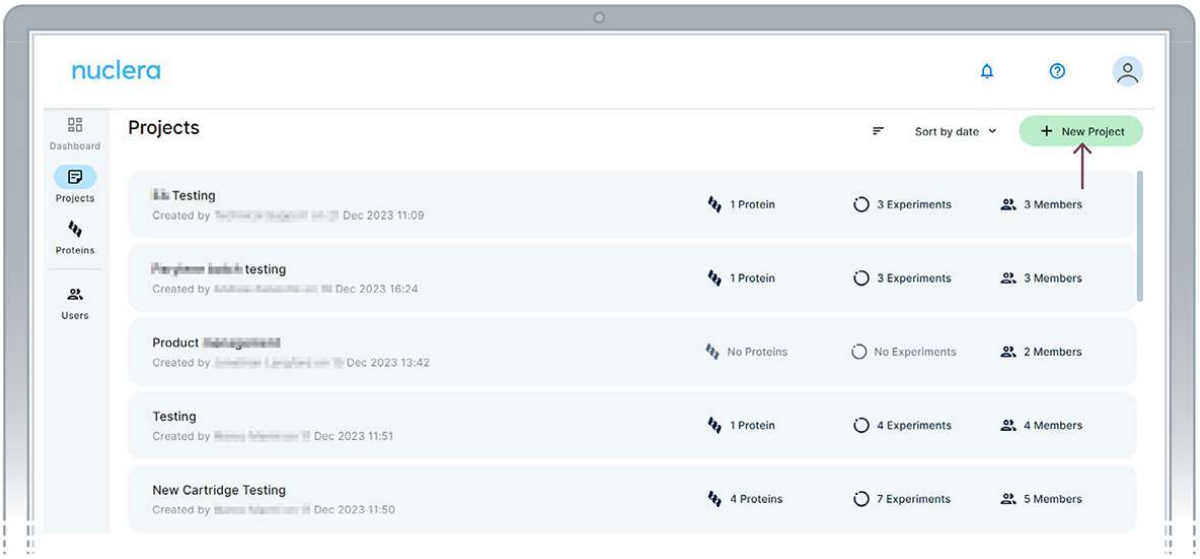
1. From the Manage Users page click the **[Invite]** button.
2. Fill the email address of the invitee and click **[Send]**.
3. The invitee will receive an email with instructions.



Set up a project - Administrator

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

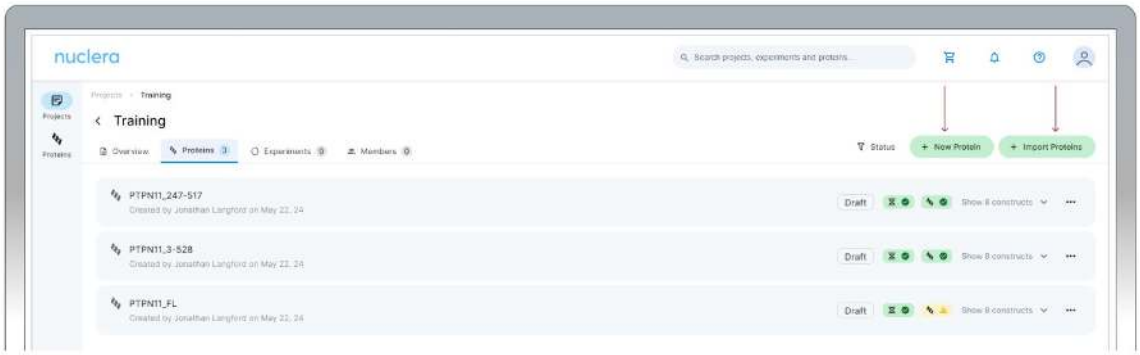
To select an existing project, click on one of the existing projects listed on the screen. To create a new project, click on the **[New Project]** button located on the top right corner of the screen.



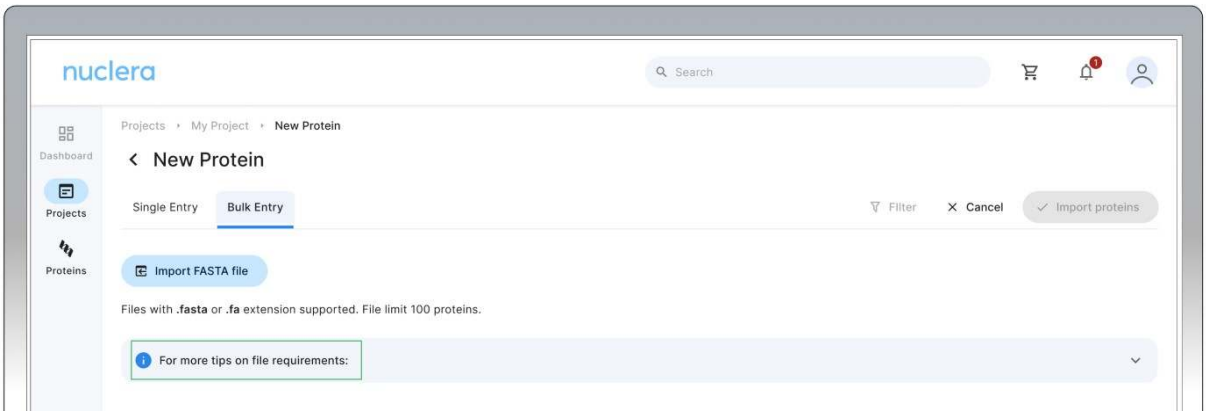
Register a protein sequence - Any user

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.

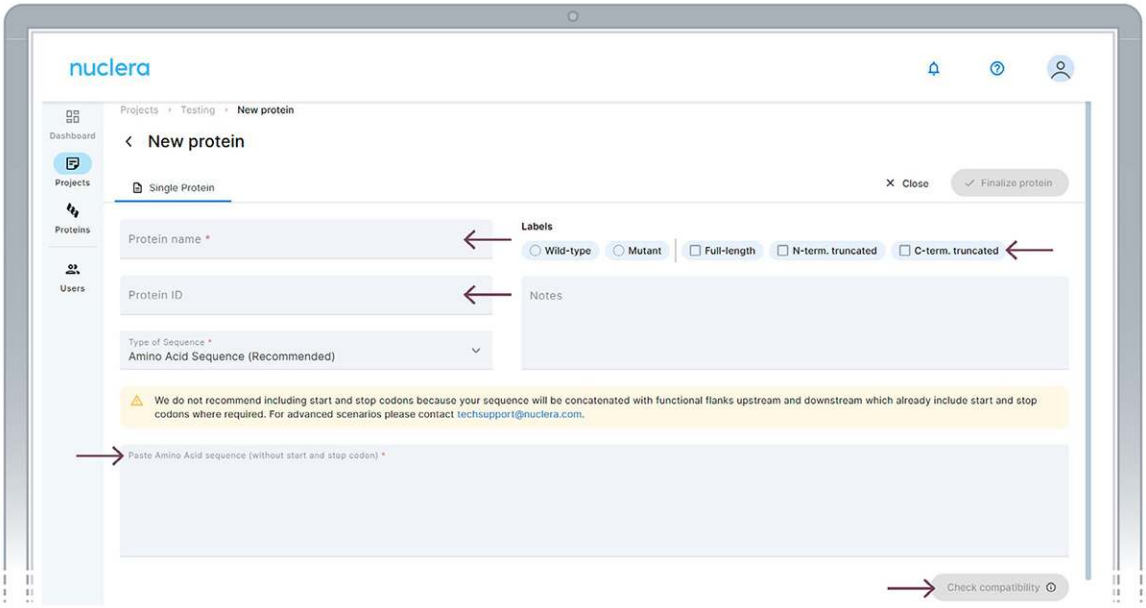


For more information about the "**Import Proteins**" feature, click on the i blue symbol



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.

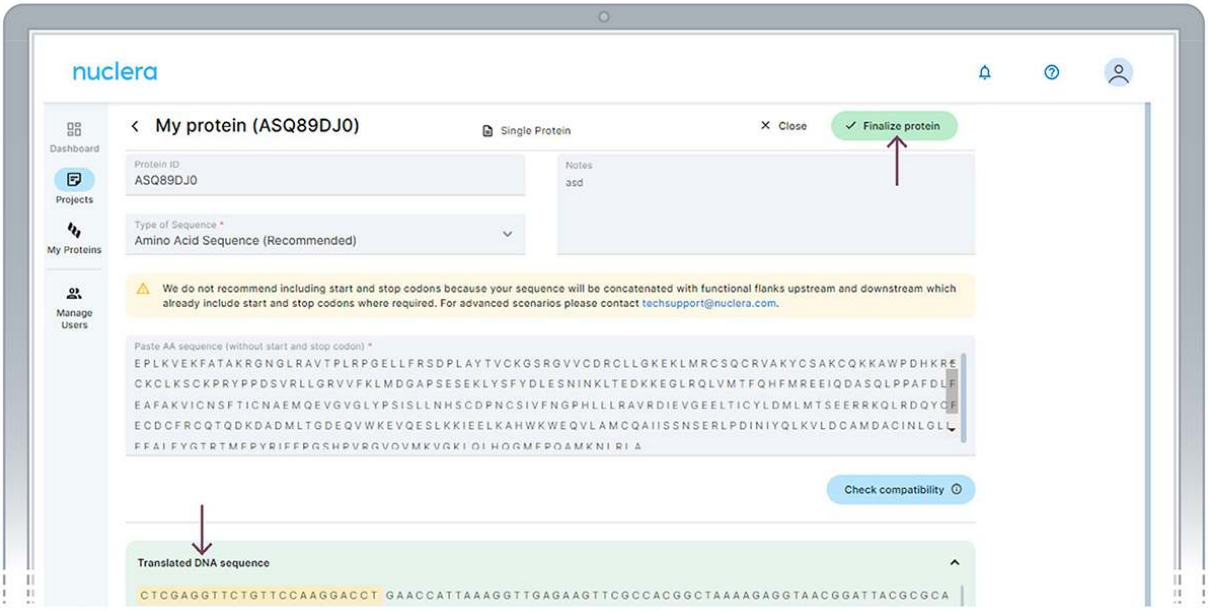


3. If you have uploaded an amino acid sequence, the sequence will be codon optimized and converted to a compatible DNA sequence. Adaptor sequences will also be added to the 5’ and 3’ end. These adaptor sequences will serve as primer annealing points during the PCR reaction to expand each construct with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

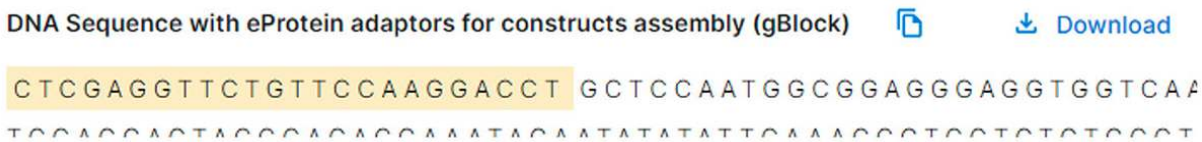
5. Protein expression compatibility checks will also be performed in the background to detect transmembrane domains, disorder regions or the presence of start and stop codons. If any expression incompatibility is detected, a warning signal will be displayed. Users can still proceed at risk or return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

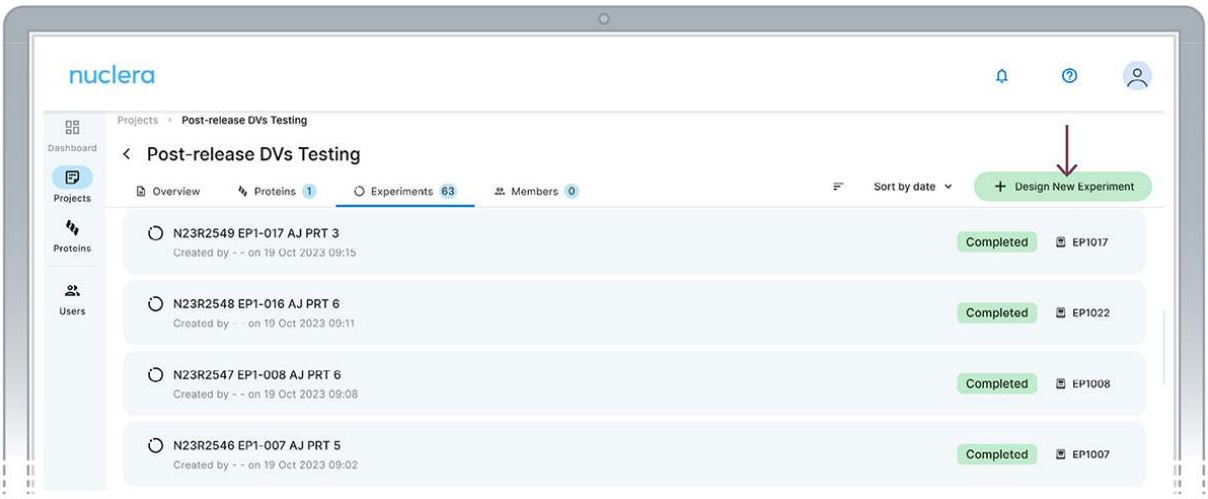
8. The translated DNA sequence can be copied and gene fragment ordered from Nuclera's recommended DNA synthesis vendor.



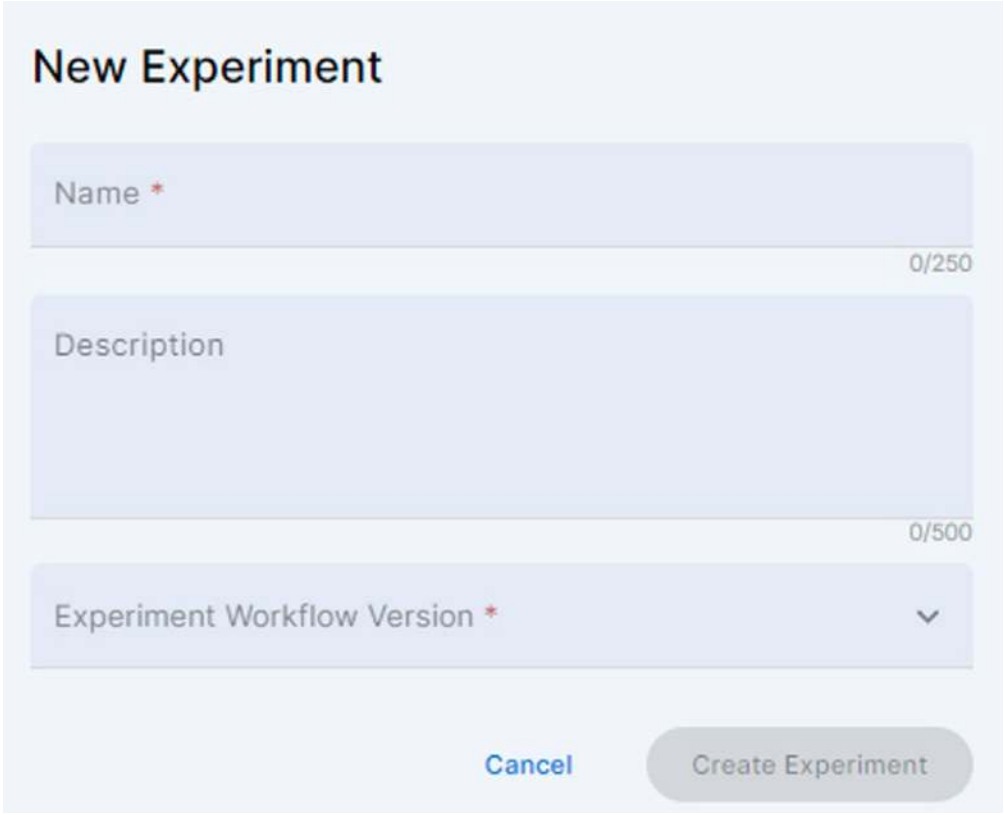
Design an experiment - Any user

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.



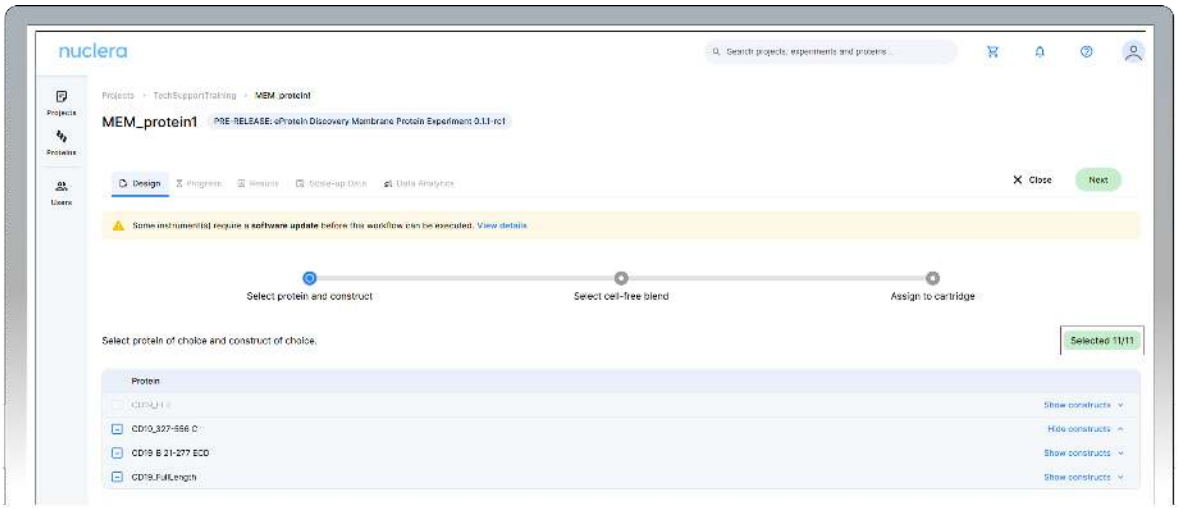
3. Enter the name of the new experiment, add a short text description and select the workflow for **Membrane Protein Screen**. Click the activated **[Create Experiment]** button



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.

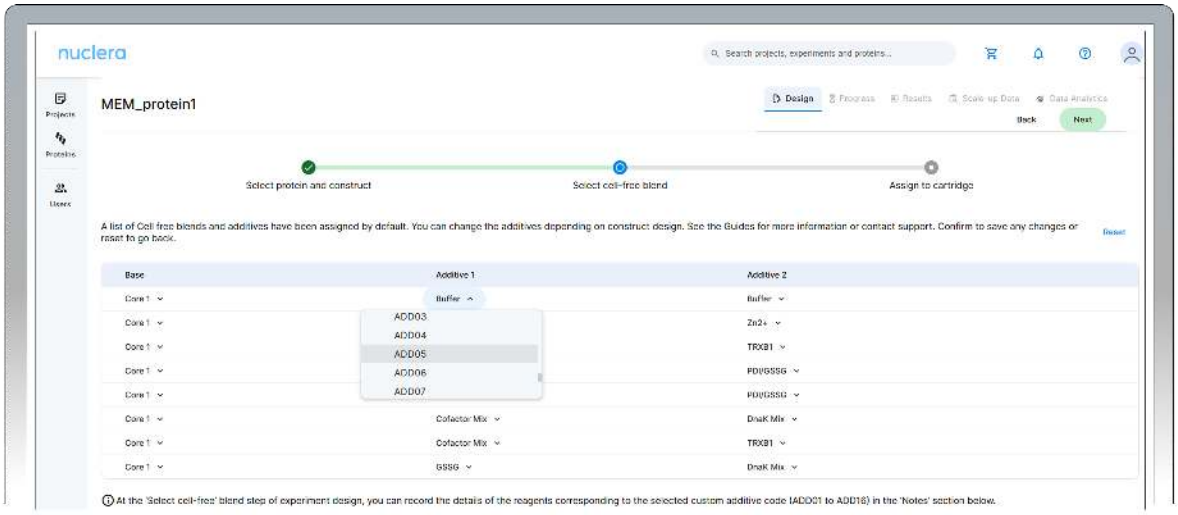


5. Select expression conditions by adding two additives to the Cell-free Core Reagent – The “Additive selection guide” sections of this document will help you to make your selection. If you are undecided on the additive combinations to use, please reach out to Technical Support. Click **[Next]** to proceed.

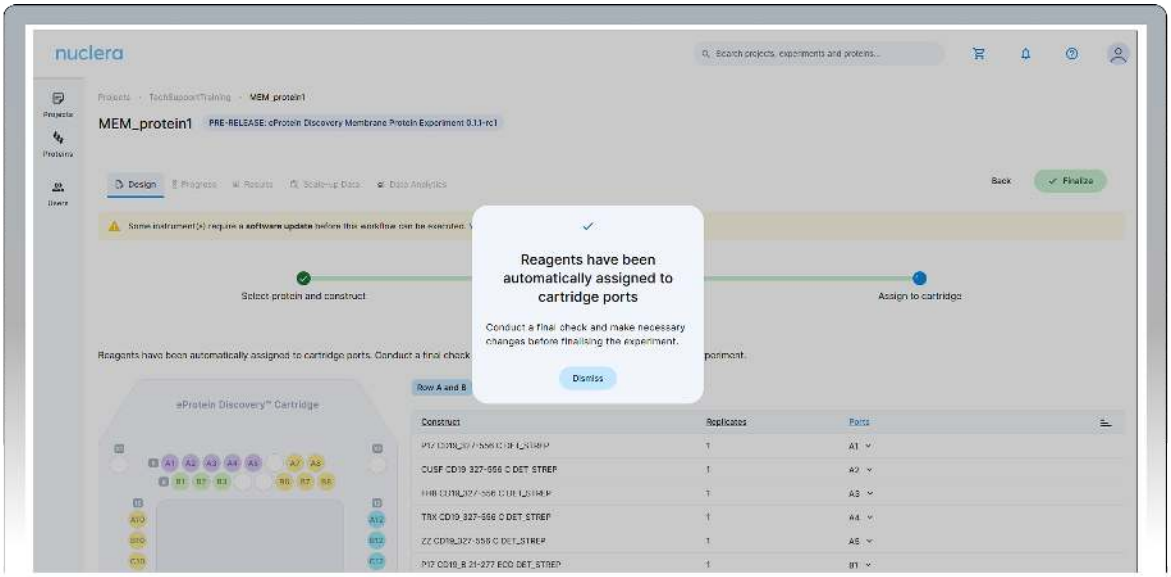
The eProtein Discovery™ system provides the flexibility to incorporate

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.

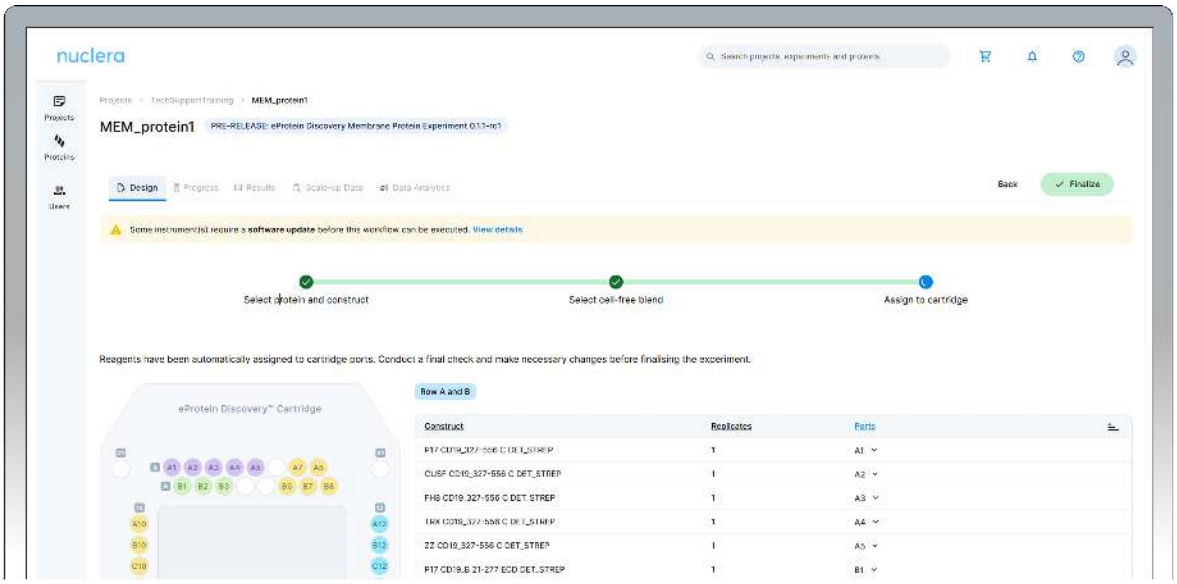
> NOTE
>
> An additive can be selected twice to enhance a specific condition.
> If using an additive that is included in our standards Cartridge Reagent Kit, use the dropdown menu to select ADD01, 02, 03 etc to distinguish between different type of nanodisc.



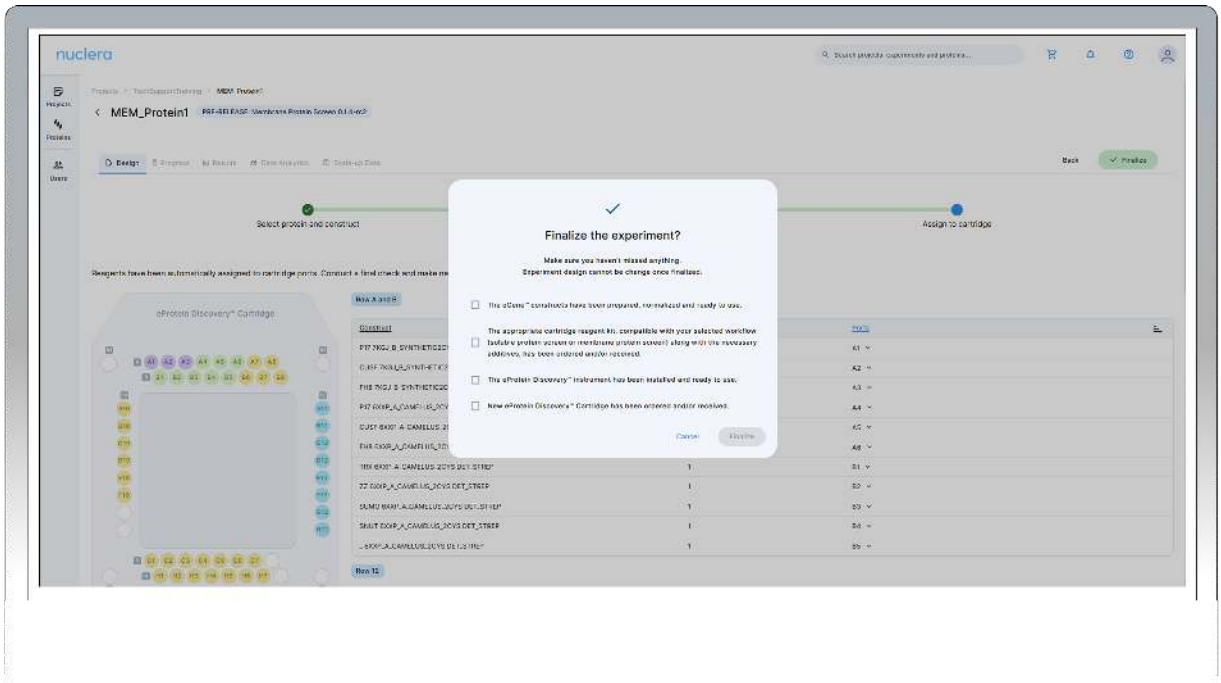
6. All samples and reagents are now assigned to a specific port on the cartridge.



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.



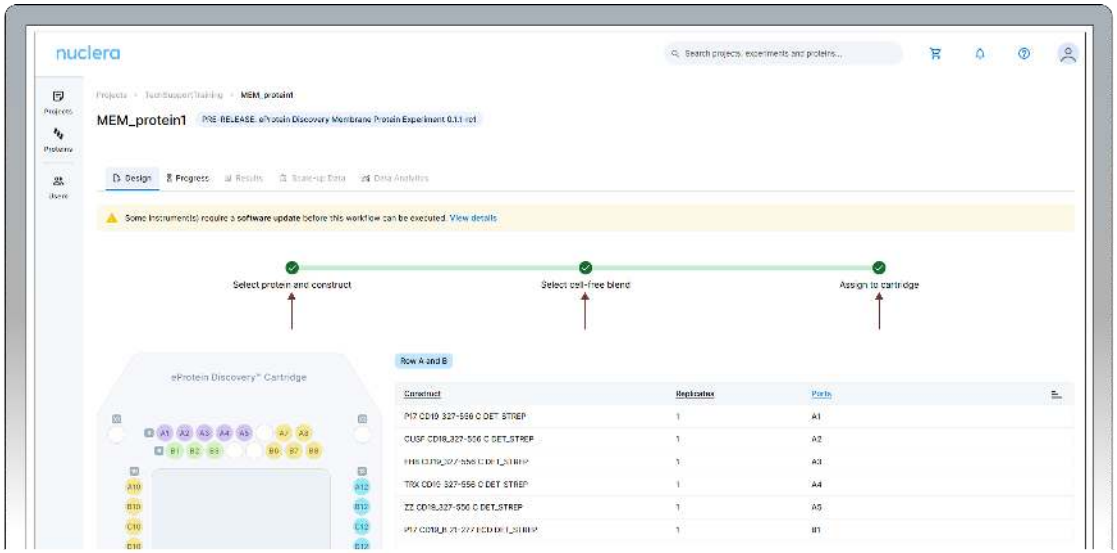
8. Press the **[Finalize]** button to proceed. This will prompt to a checklist to make sure everything is ready for the experiment.



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).

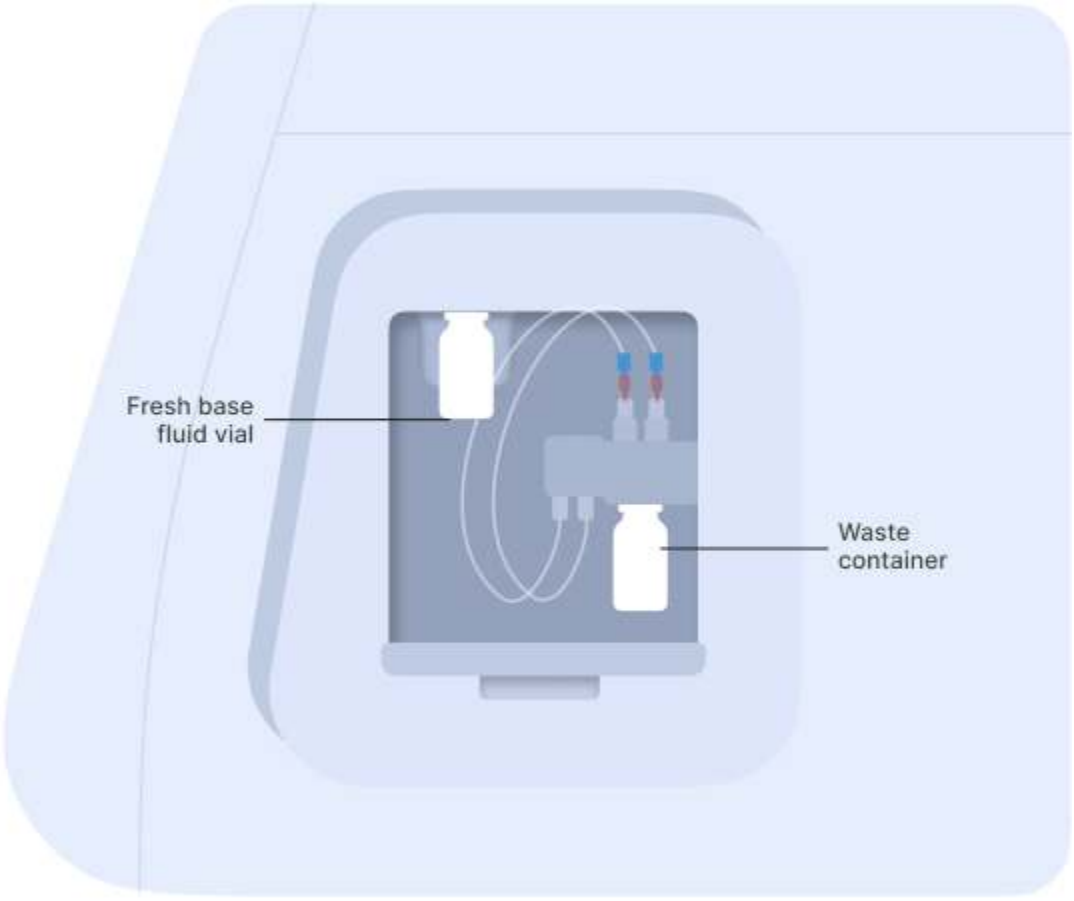


Figure 3: Vial of base fluid and the waste container connected to the pump as shown on the screen

[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.

Note: If a eGene construct is missing it must be substituted with 5 µL of Blank Buffer.

IMPORTANT NOTE

Do not substitute a missing eGene construct with water.

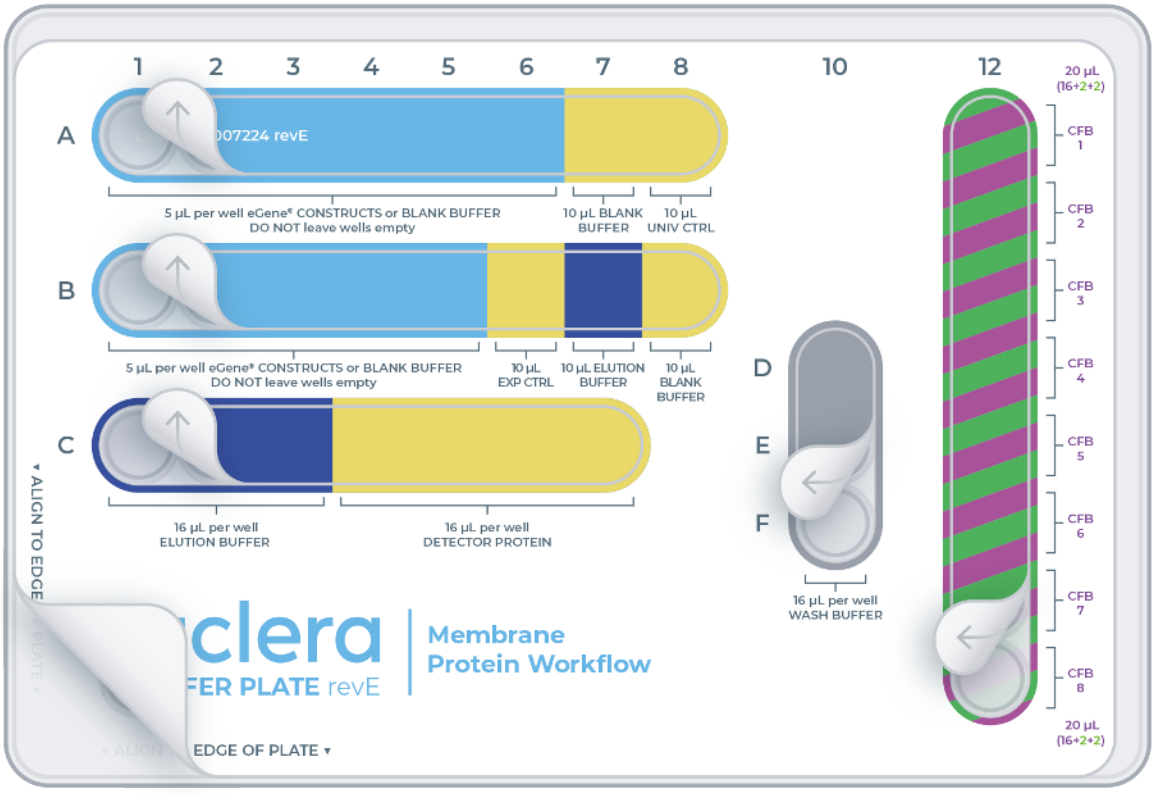


Figure 5: Transfer plate layout

Reagent	Volume (µL)
eGene construct	5
Controls: Blank Buffer, Universal Control (Univ. Ctrl), Expression Control (Exp Ctrl)	10
Cell-free Blend (CFB): Cell-free Core Reagent + Additive	20 (16+2+2)

Reagent	Volume (µL)
1 + Additive 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.

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).

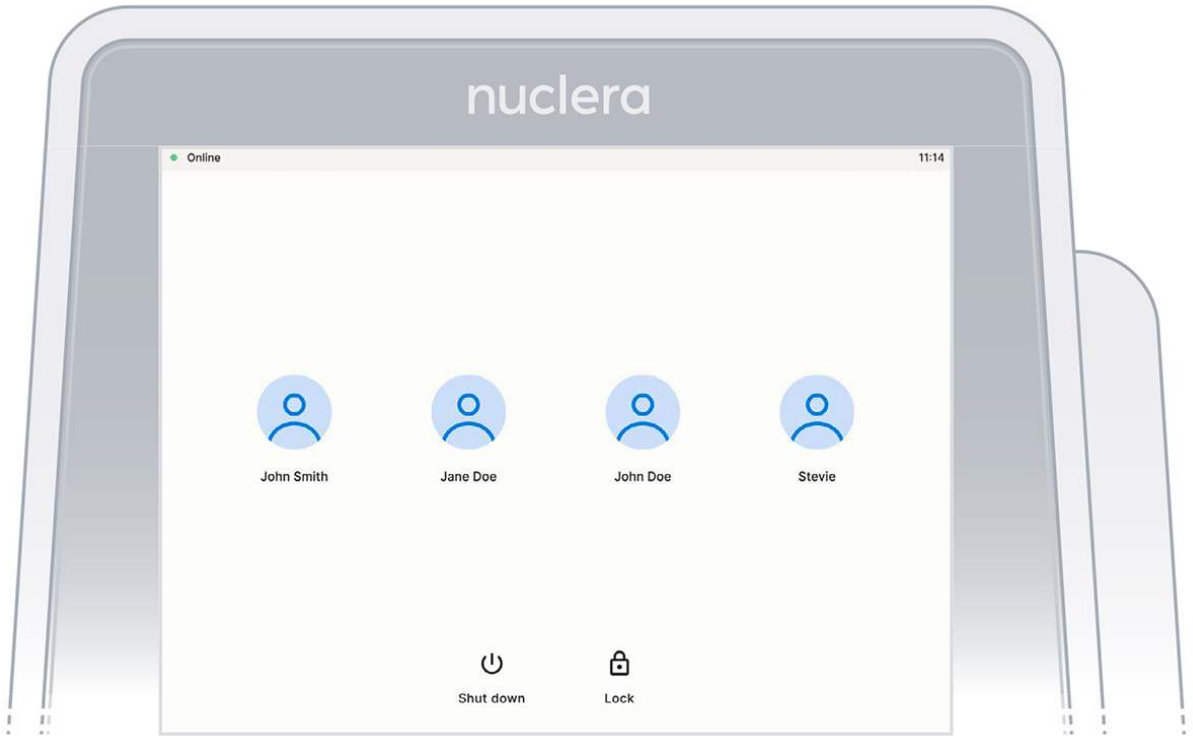


Figure 6a: User accounts on the instrument software

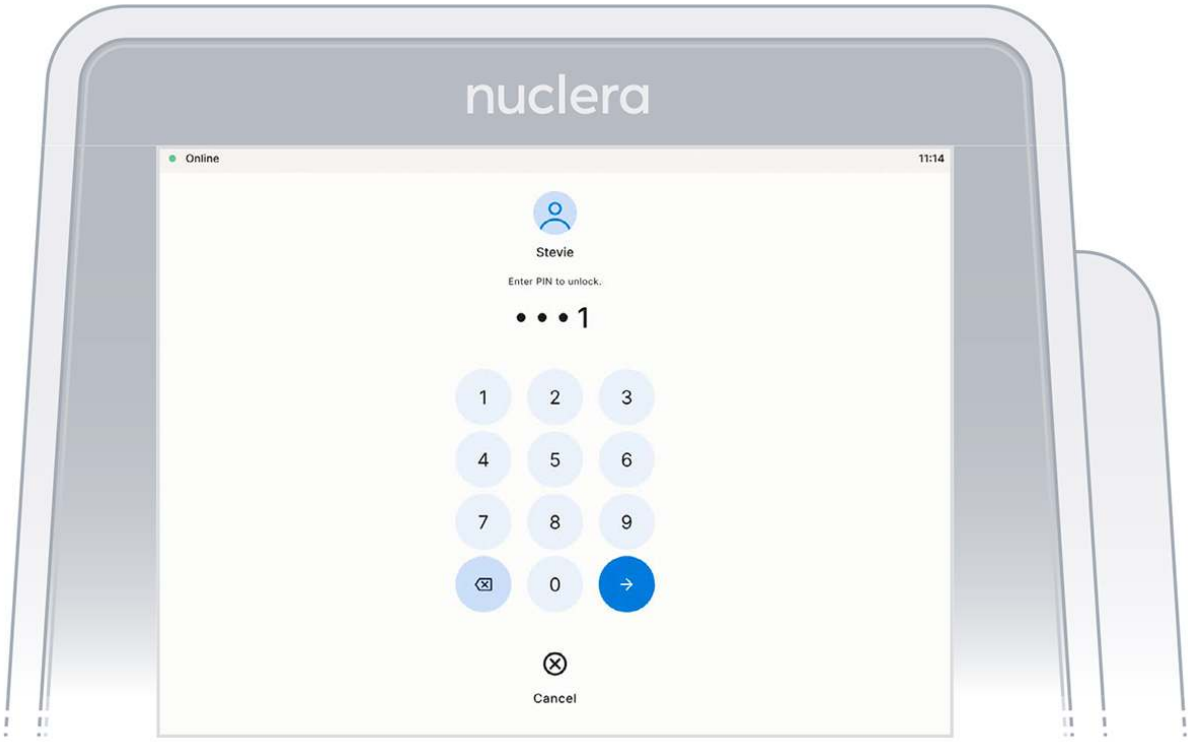


Figure 6b: PIN Interface

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

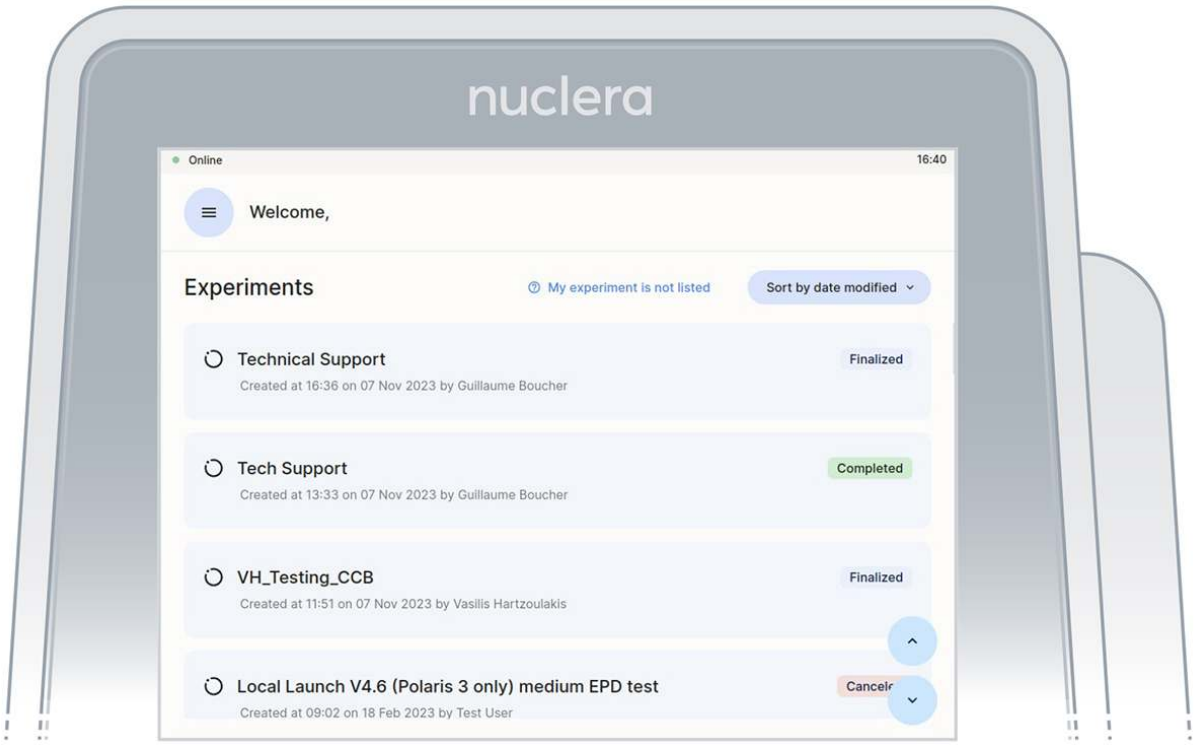


Figure 7: Instrument software welcome page with the list of finalized experiments

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

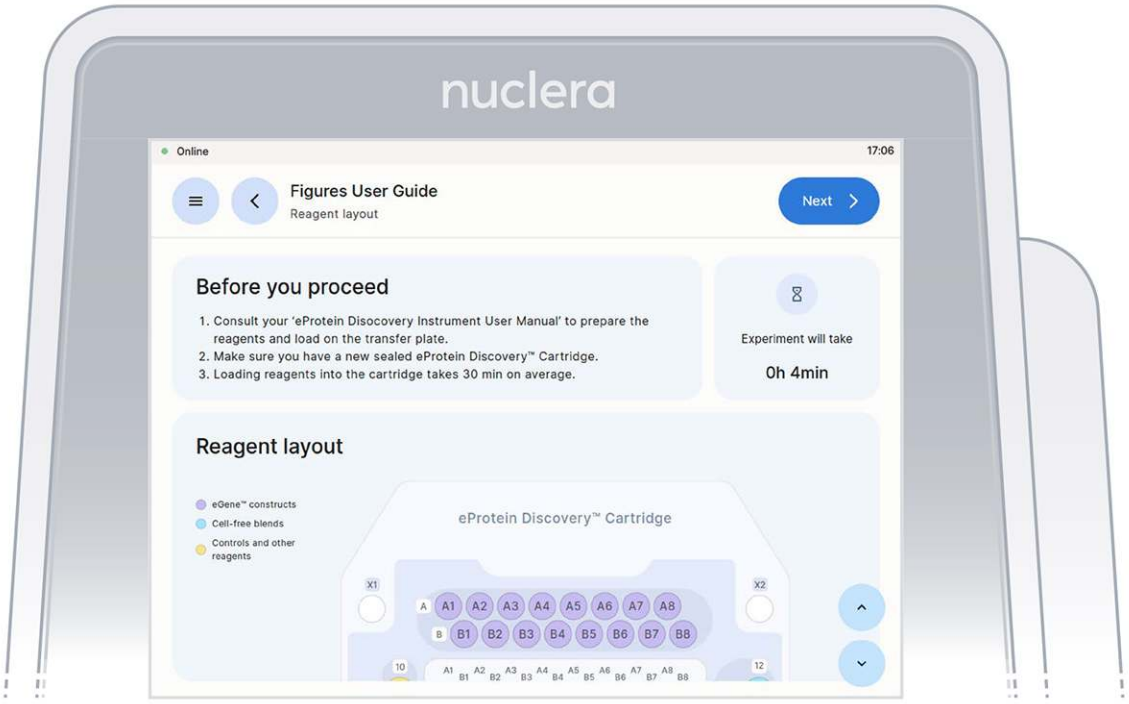


Figure 8: Instrument software welcome page with the list of finalized experiments

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

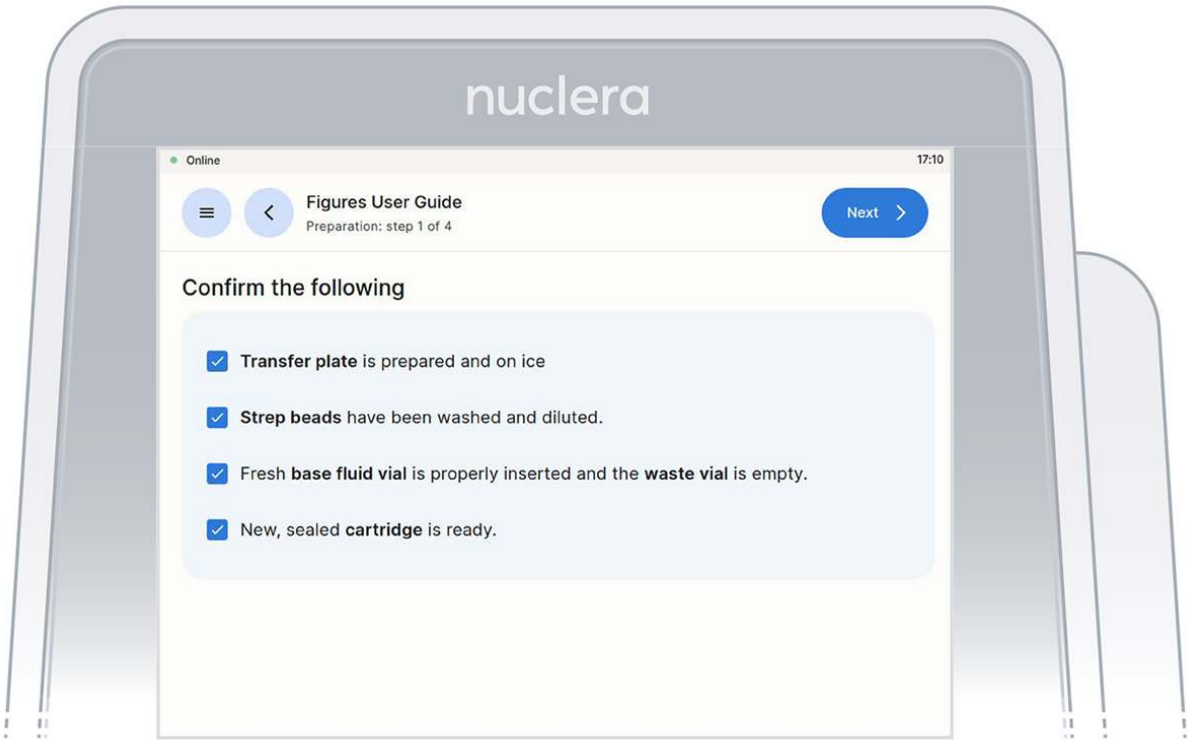


Figure 9: Loading of the cartridge on the eProtein Discovery instrument.

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).

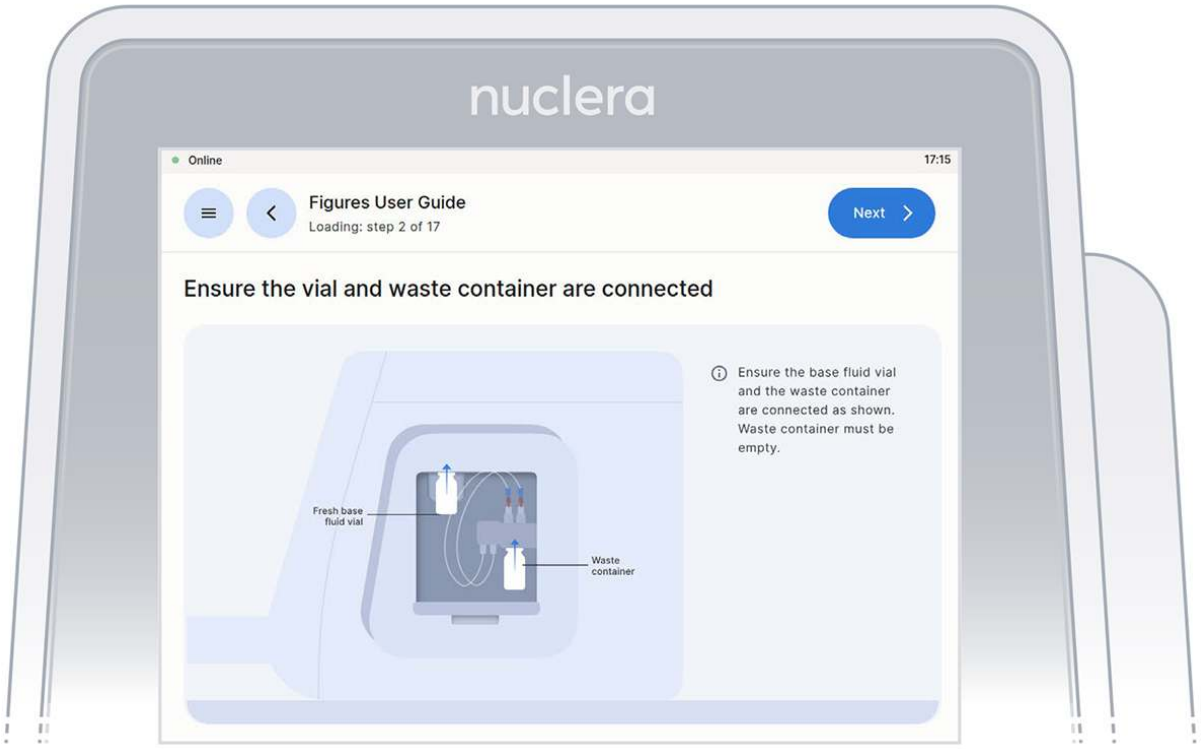


Figure 12: Vial of base fluid and the waste container connected to the pump as shown on the screen

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.

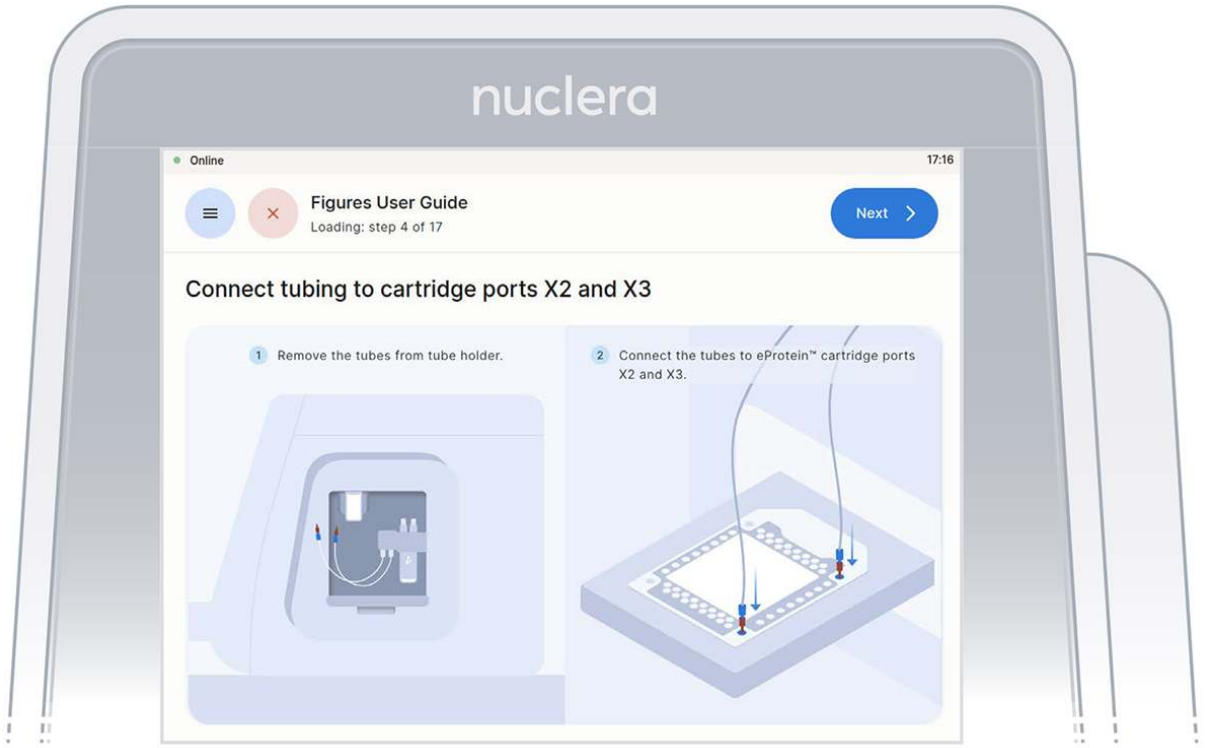


Figure 14: Inspection that all the ports on the cartridge are filled with base fluid

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).

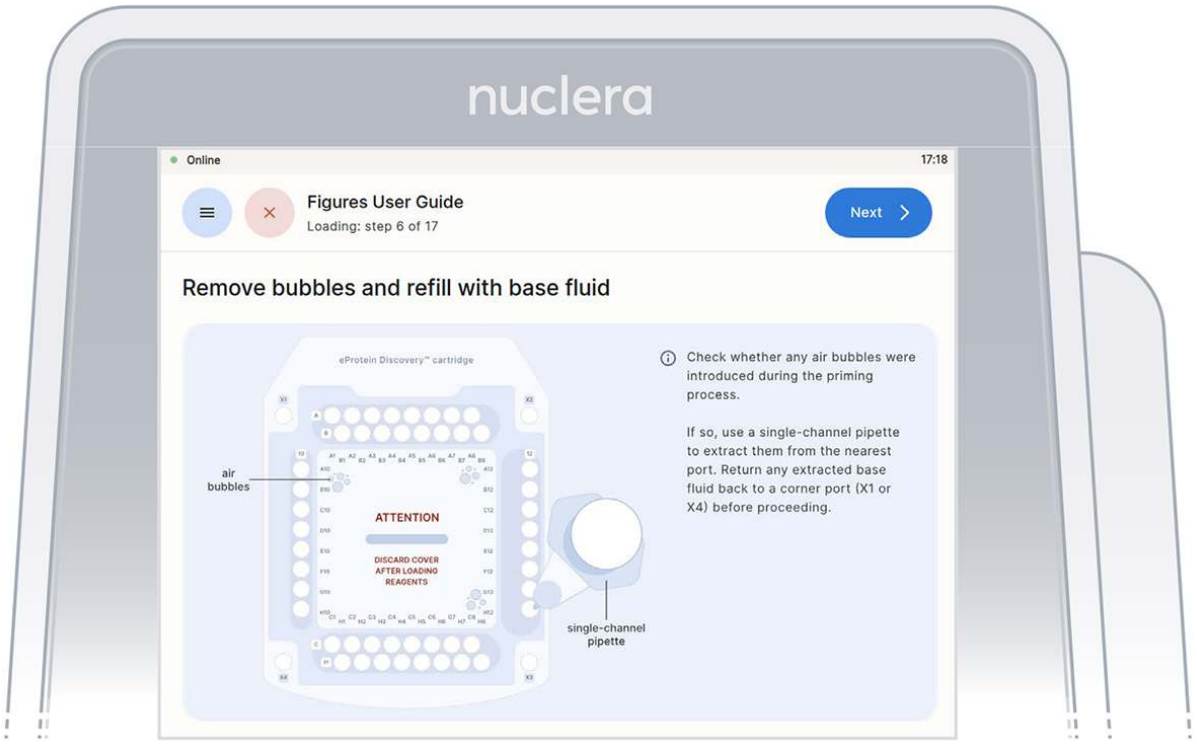


Figure 15: Connection of the pump tubes to the cartridge

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).

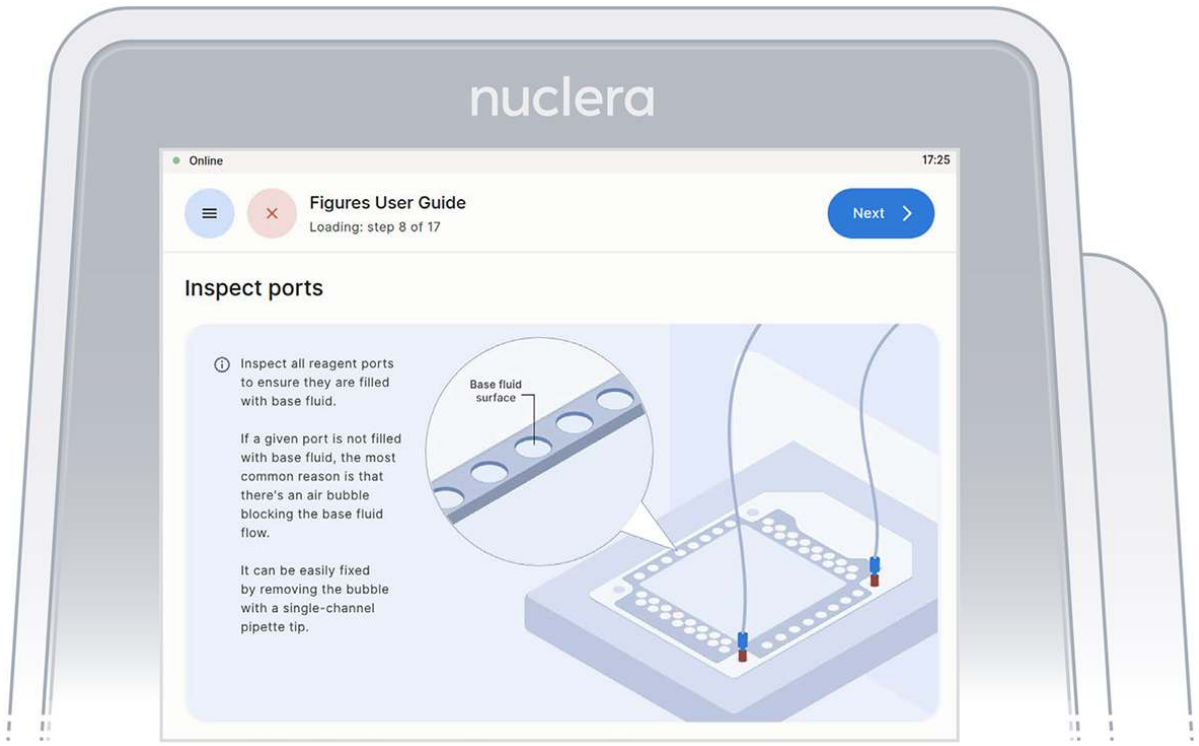


Figure 16: Confirm that all the ports on the cartridge are filled with base fluid

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

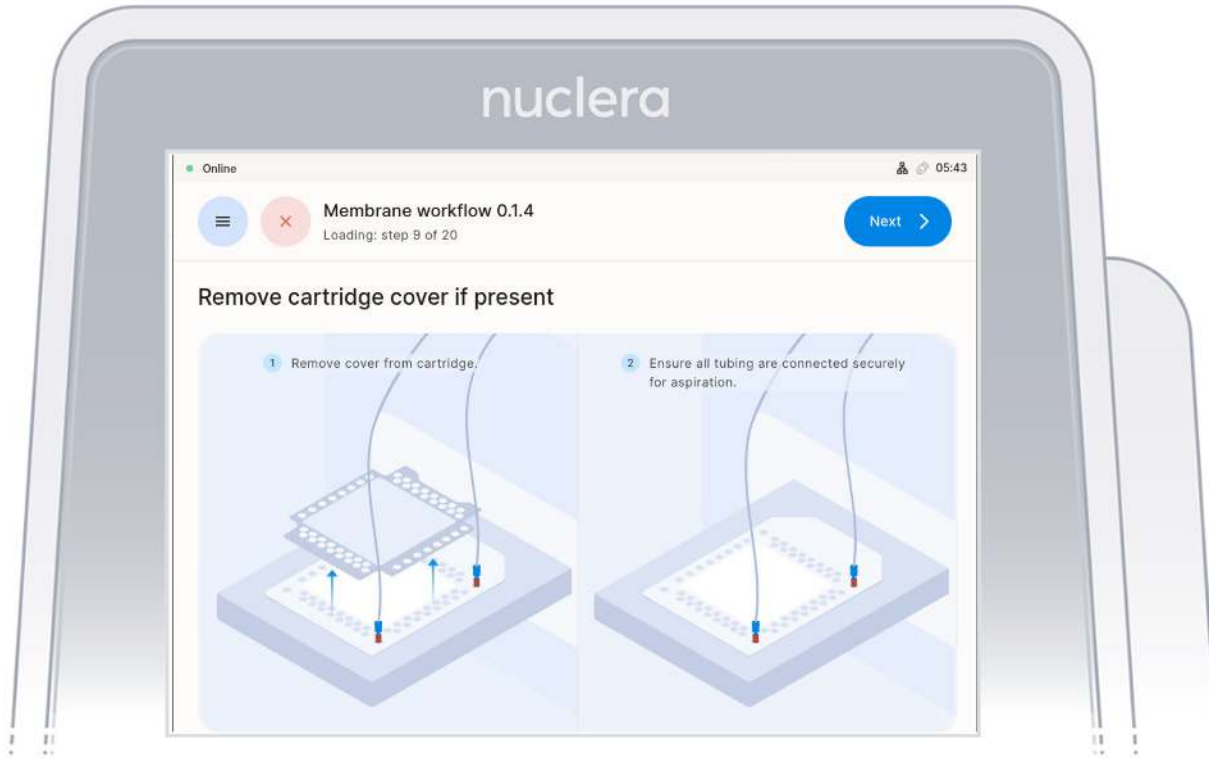


Figure 17:Remove cartridge cover

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

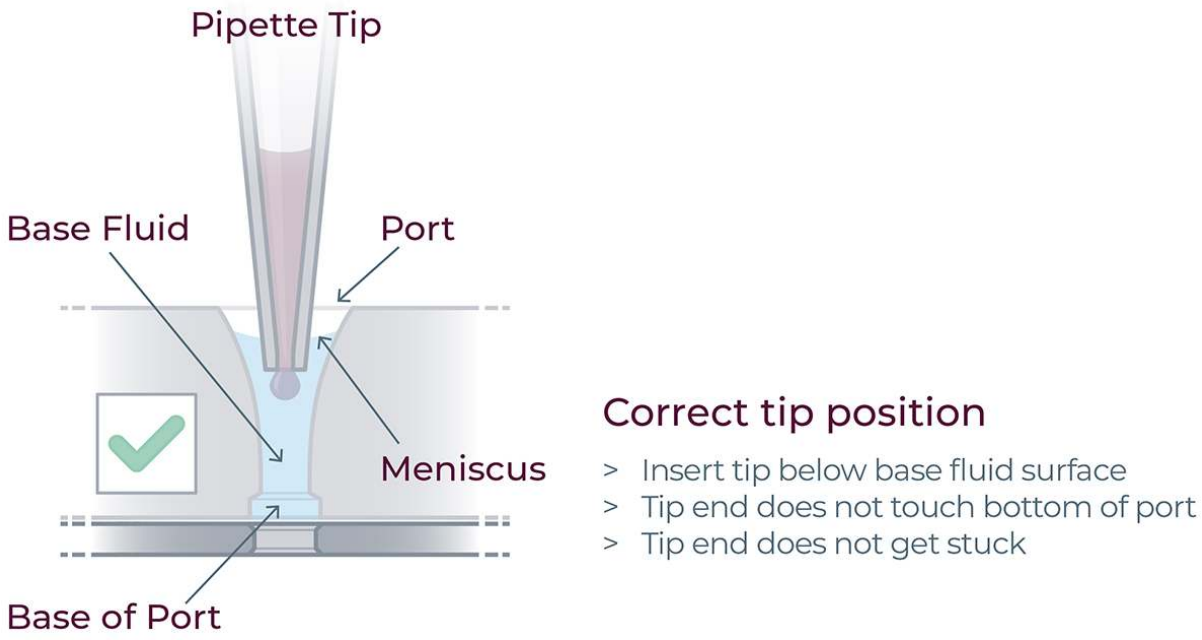
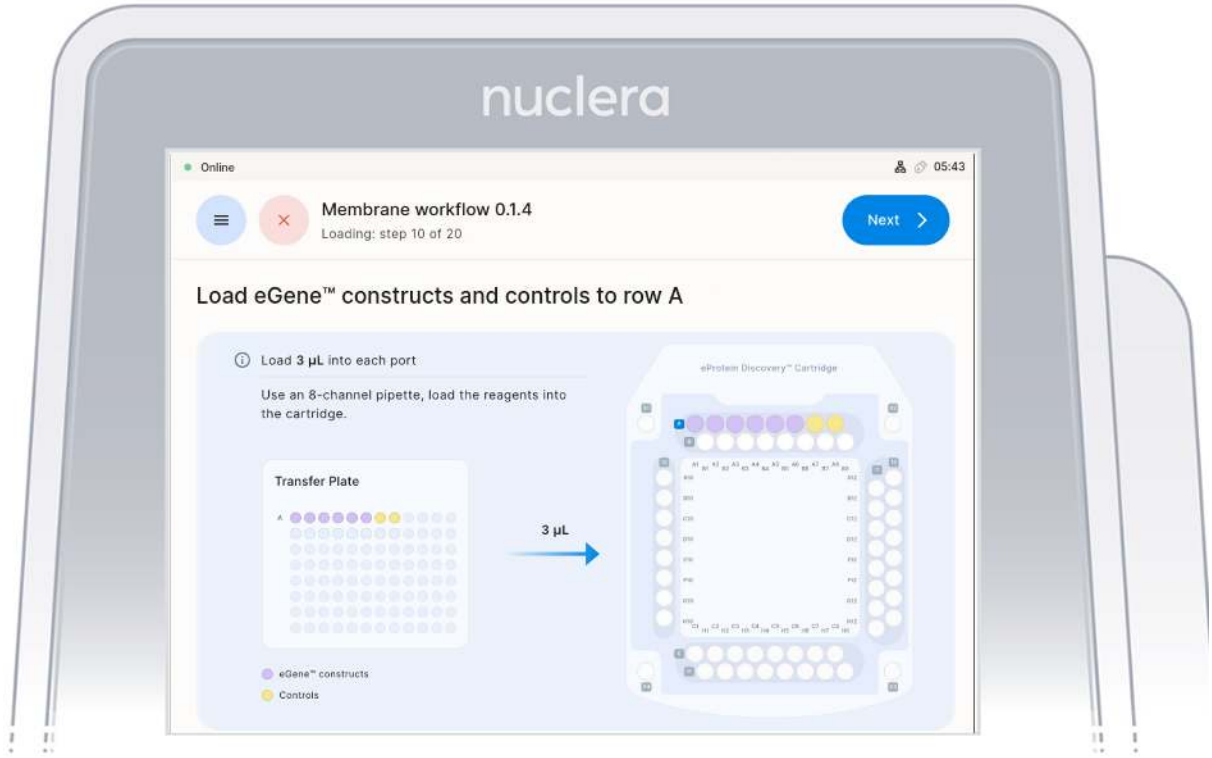


Figure 17: For correct reagent loading the pipette tip is immersed in the base fluid and not touching the bottom of the port

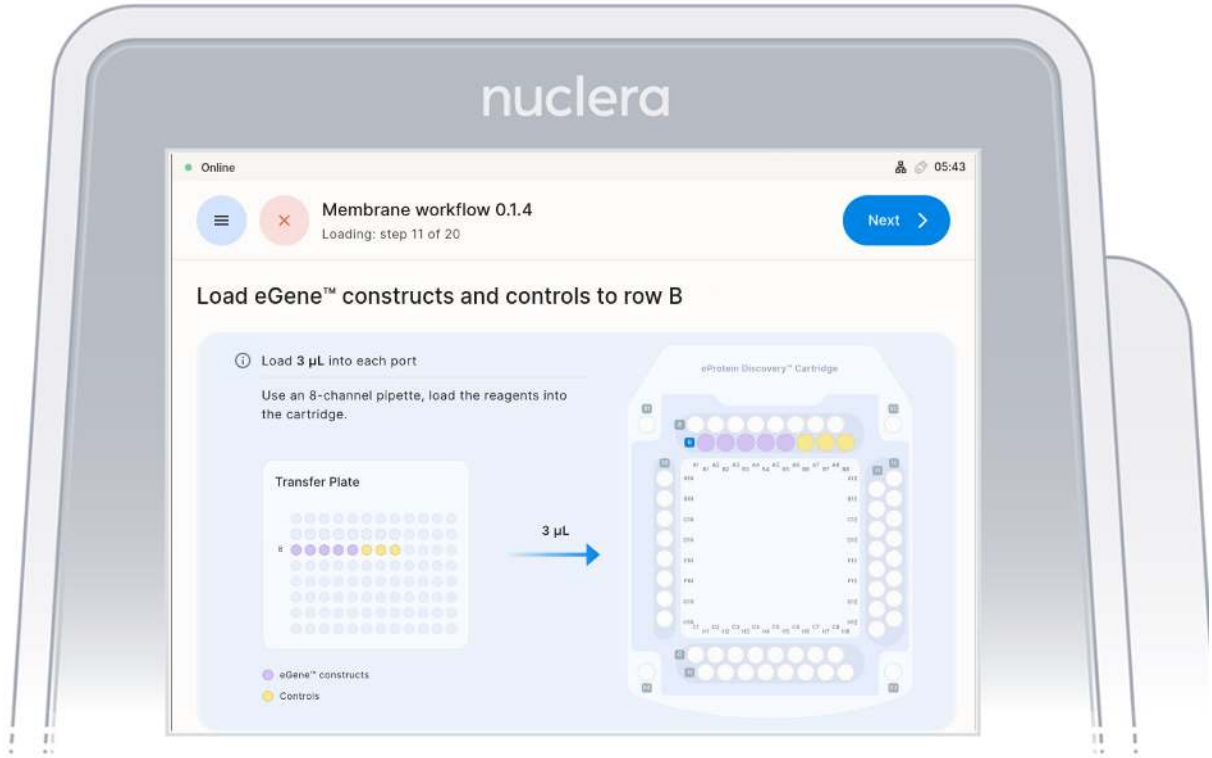
Load reagents

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.



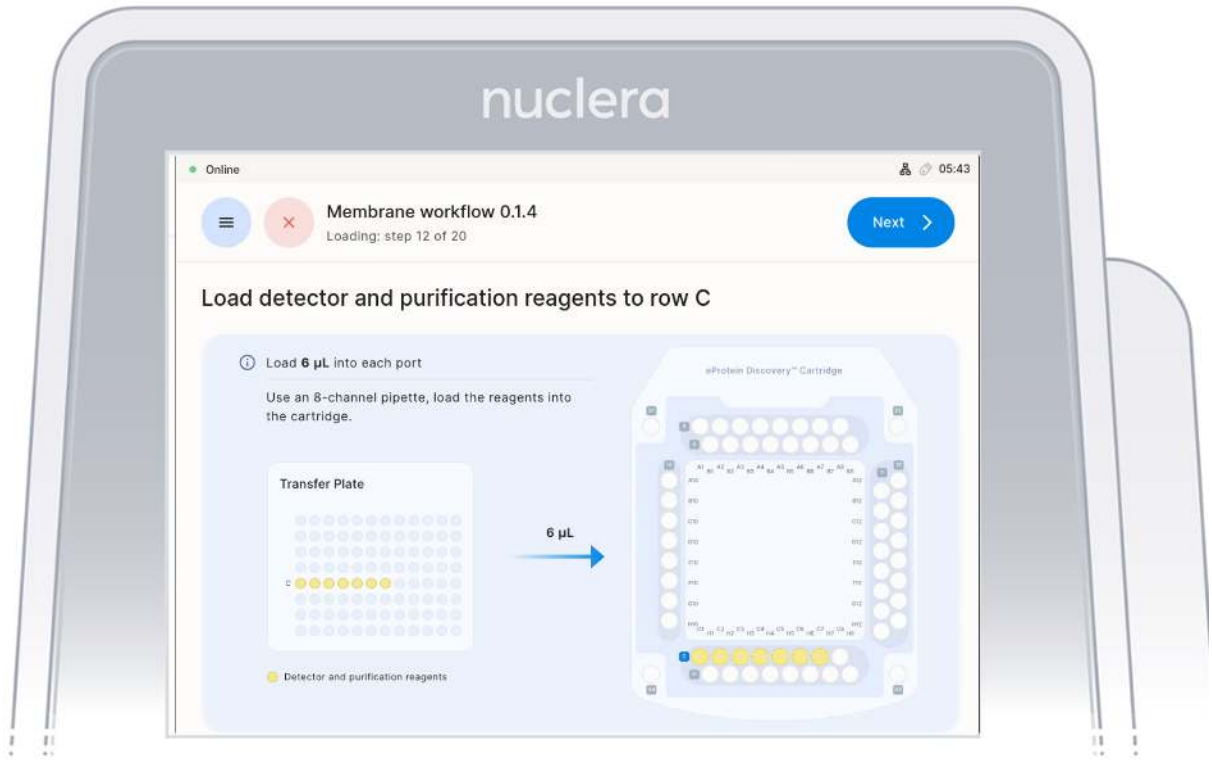
3. Reagents - row C:

▹ 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.



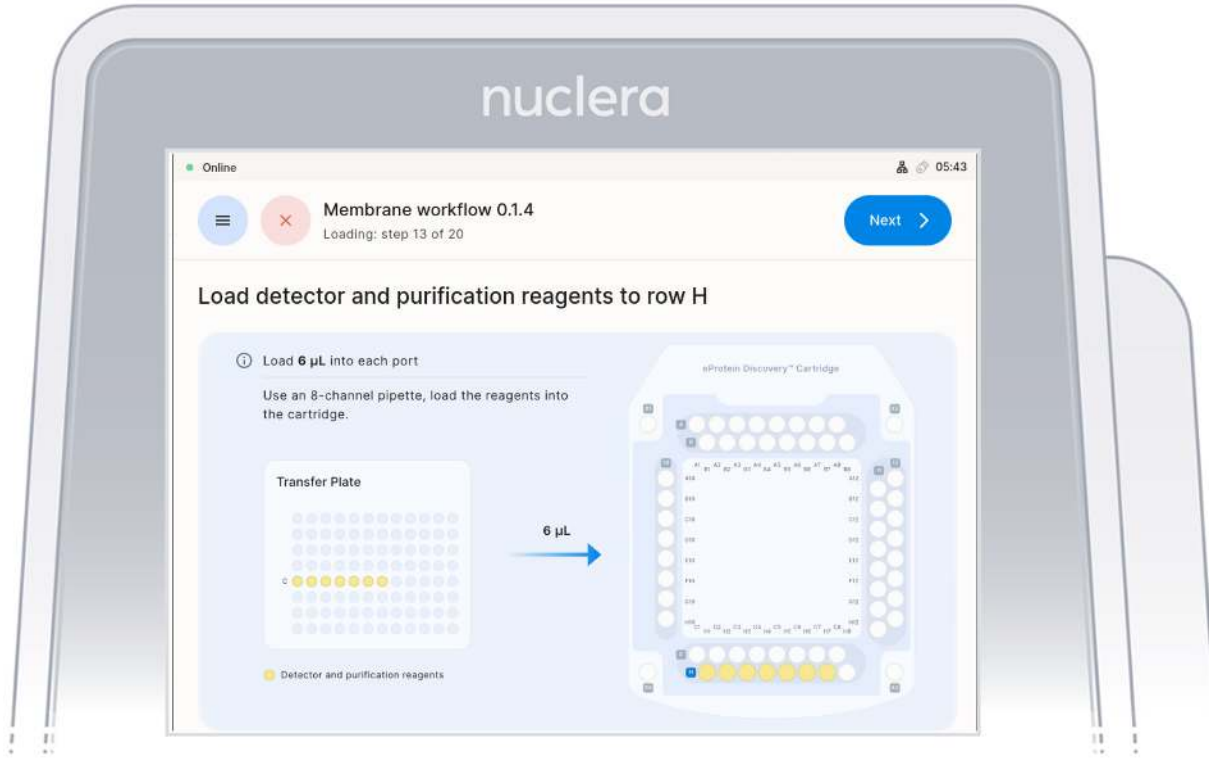
4. Reagents - row H:

▹ 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:

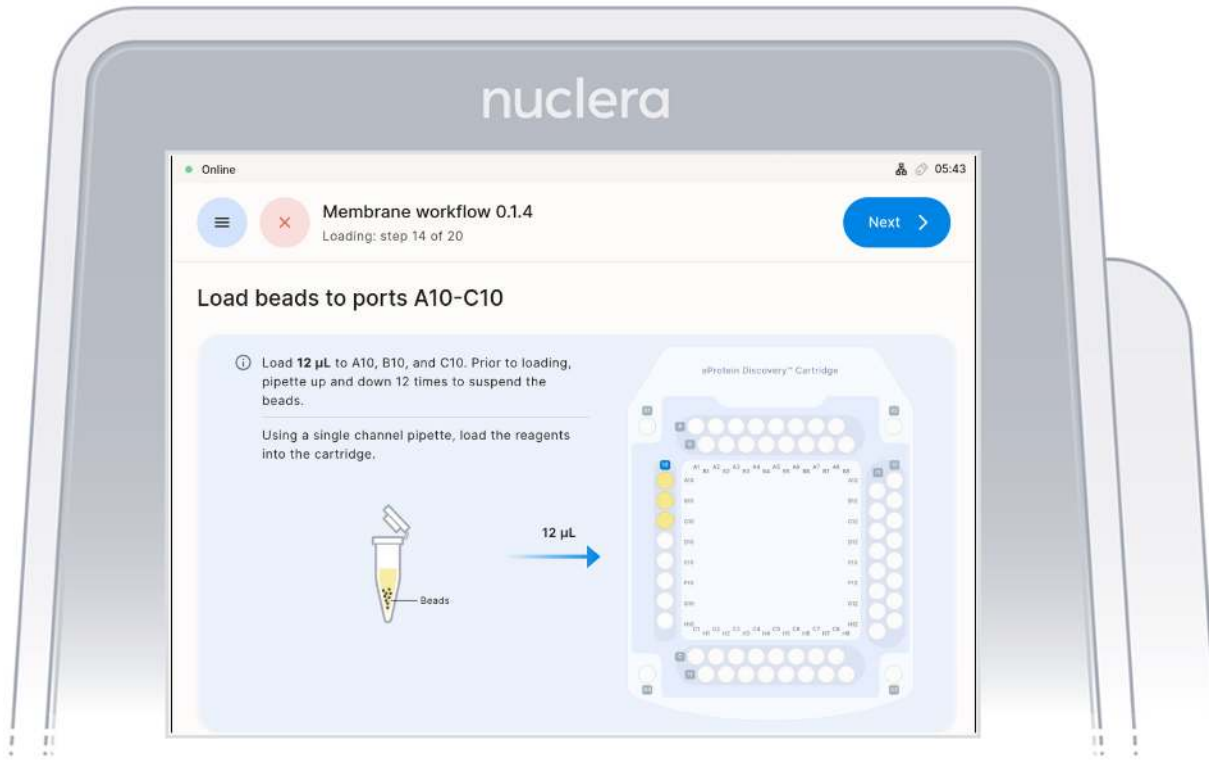
▹ Using a single-channel P20 pipette, mix the first Strep Purification Beads tube by gently pipetting up and down 12 times, avoiding air bubbles. Immediately aspirate 12 µL and dispense into port A10 of the cartridge. Repeat the same process for the second and third tubes, dispensing into ports B10 and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

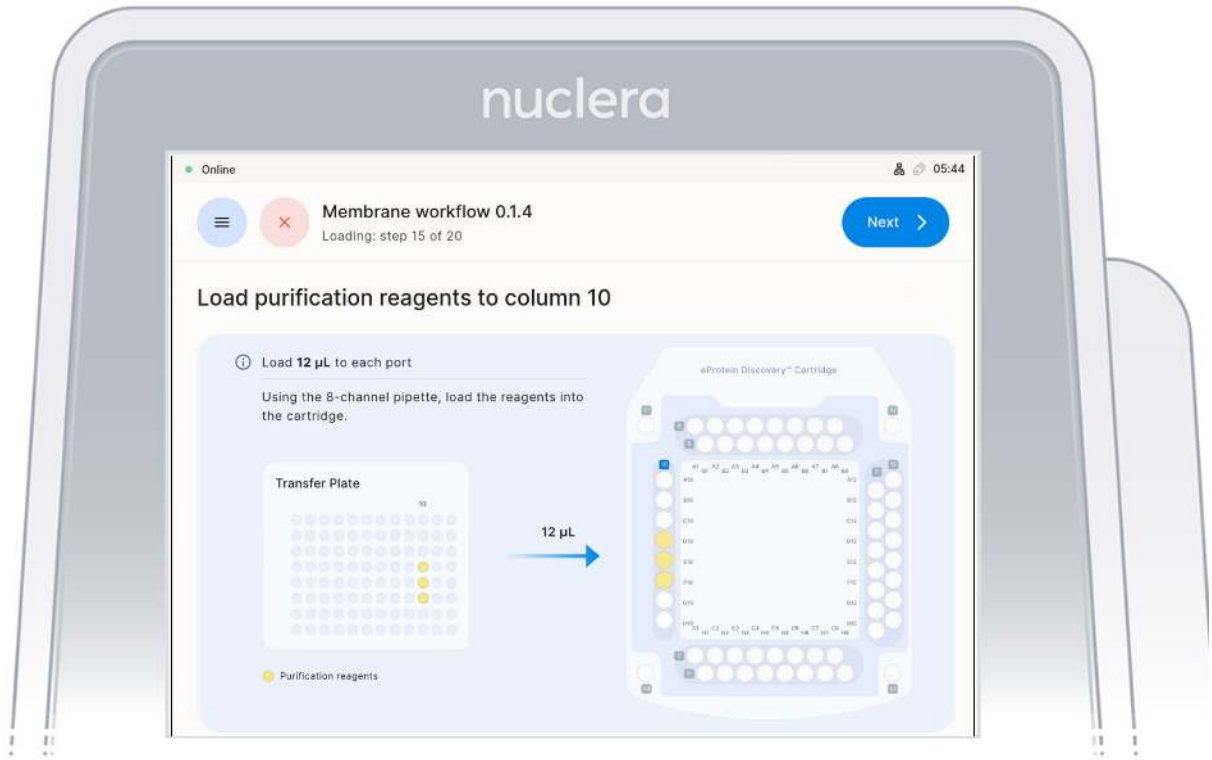
▹ Eject the pipette tip into a waste container.

▹ 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. **

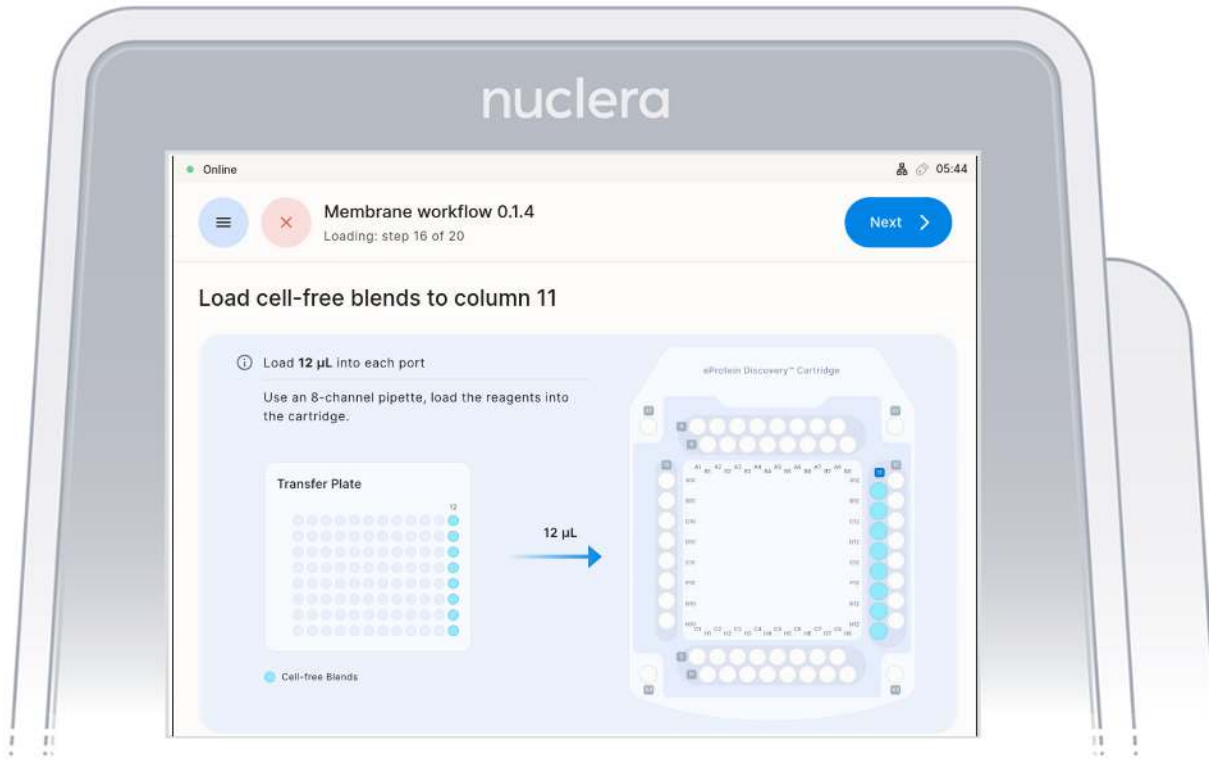
> IMPORTANT NOTE
>
> Be careful not to introduce air bubbles in the ports.
> Aspirate 12 µL of the Cell-free Blends from the transfer plate wells

▹ 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).

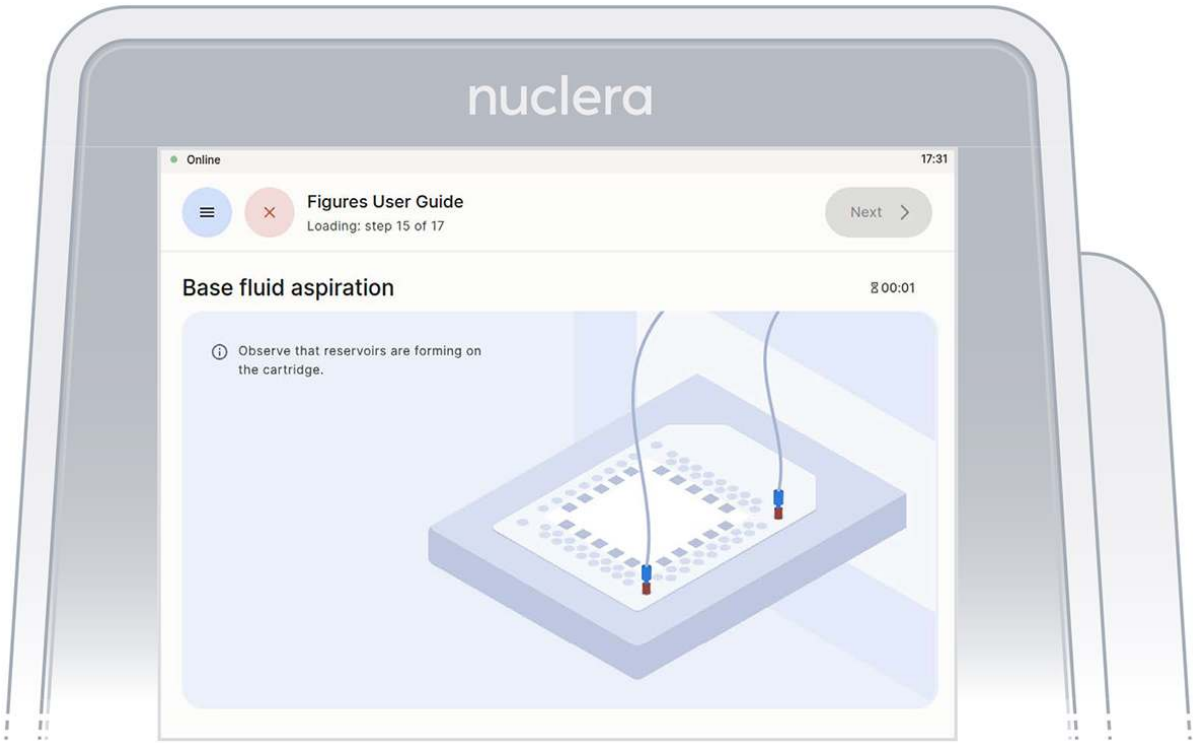


Figure 24: Base fluid aspiration

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.

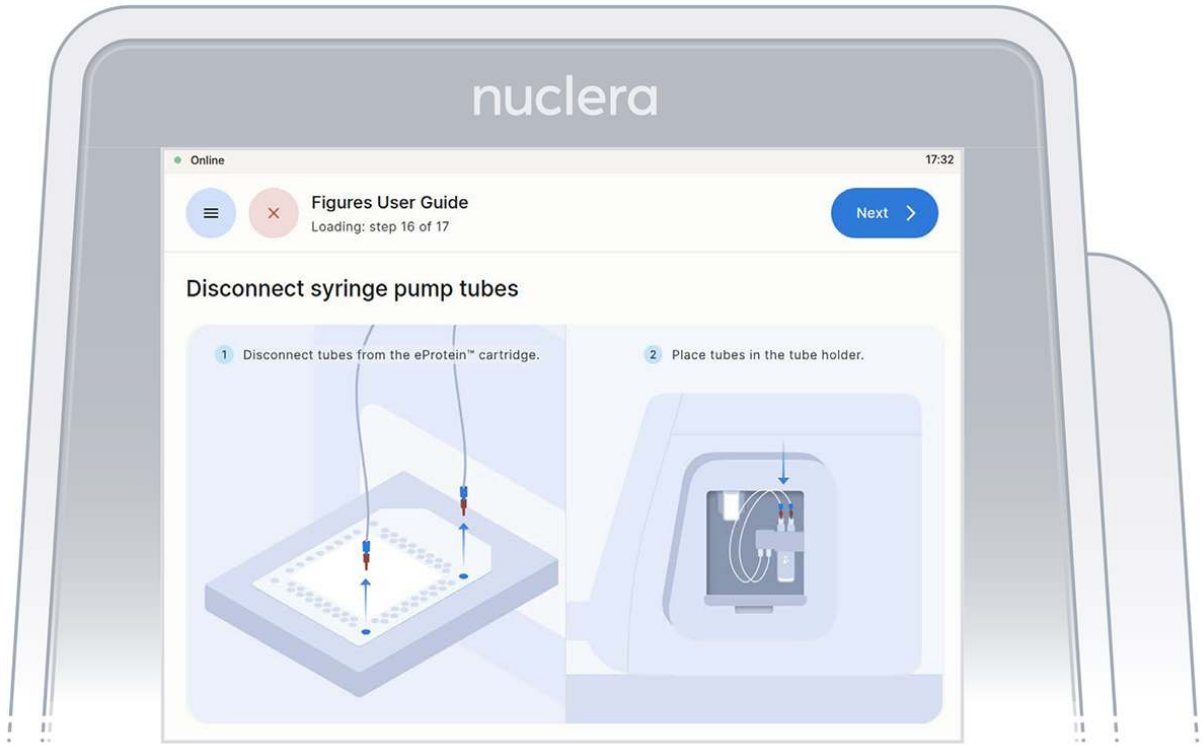


Figure 25: Disconnect the tubes and place them on the tube holder

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.

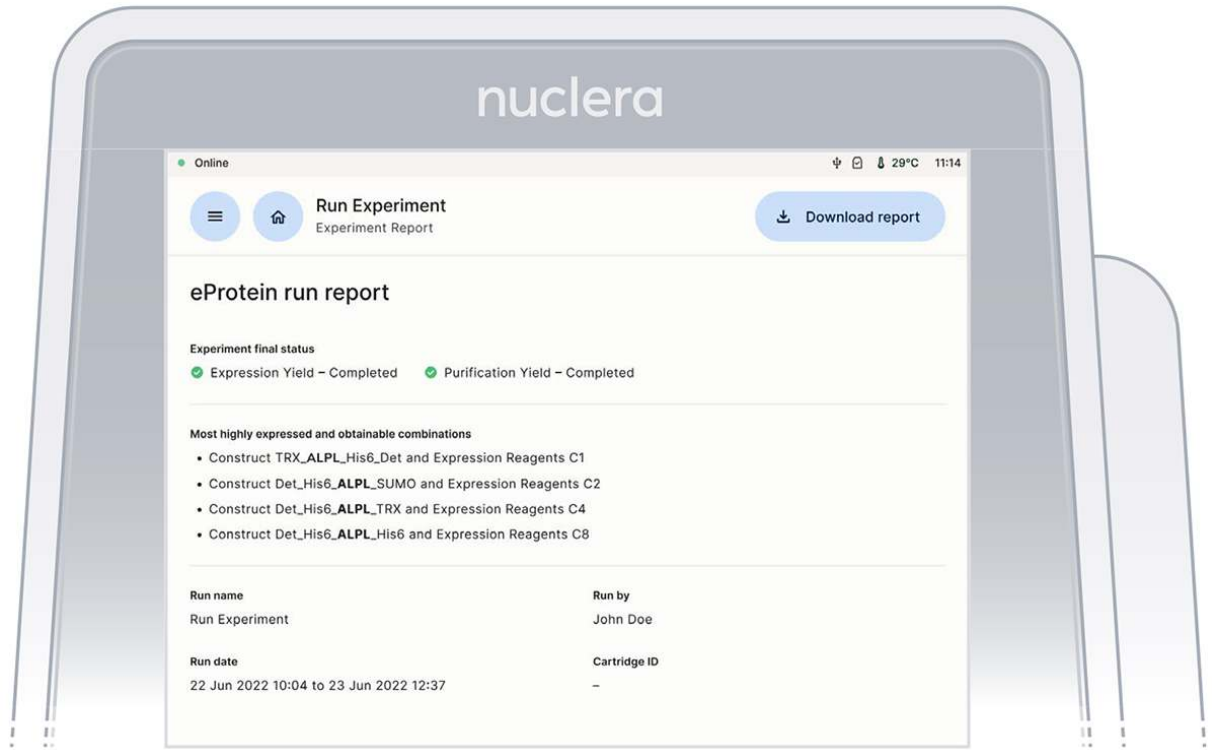


Figure 28: Result screen from the Instrument Software

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)

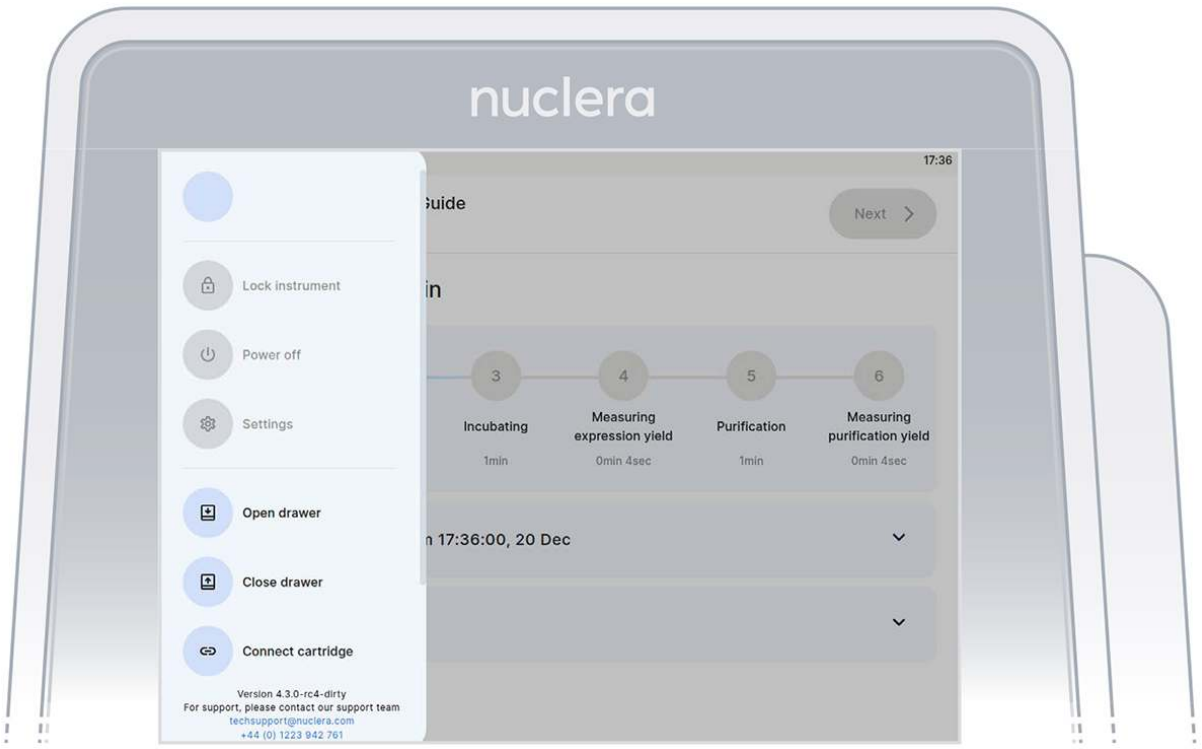


Figure 30: Remove the cartridge from the instrument

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!

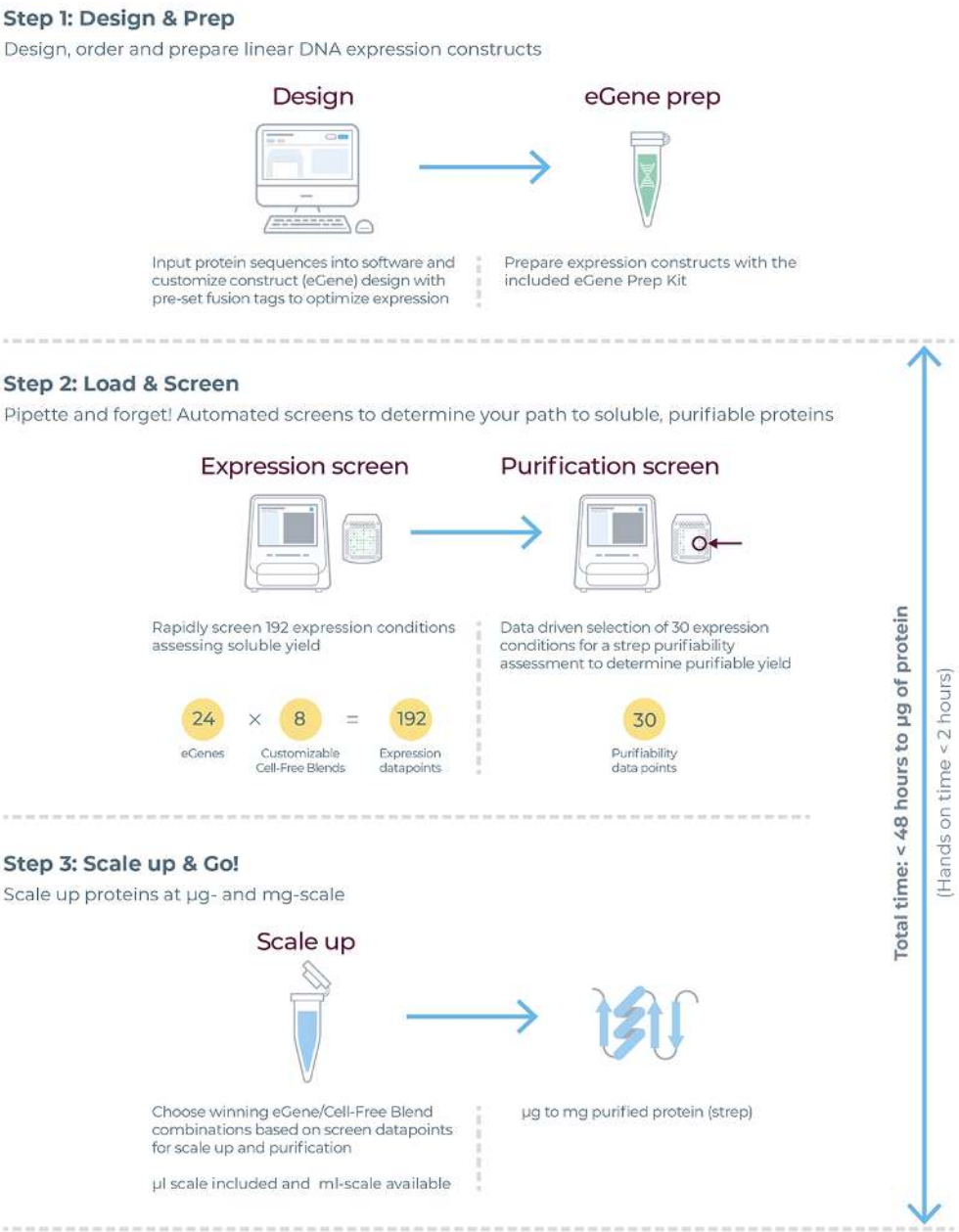
Software: eProtein Discovery™ software simplifies a complex multivariate experimental design. The software sets up and simultaneously tracks 192 different combinations of DNA sequences, flank pairs and expression reagent reactions performed on eProtein Discovery™ system. AI performs highly rigorous QA 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. • 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.

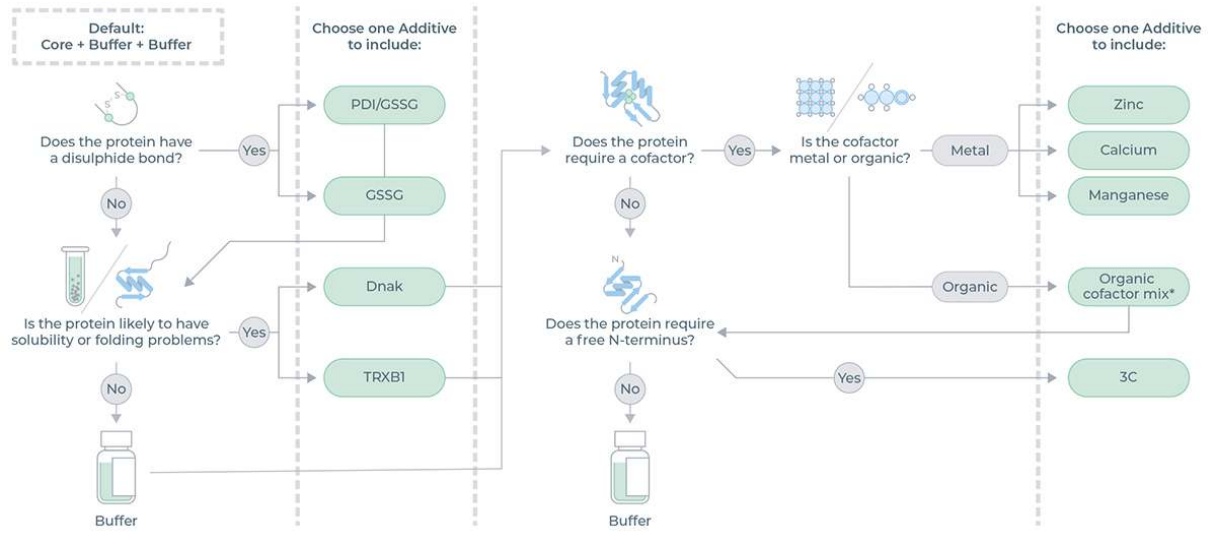


Figure 1: Decision tree which illustrates the Additive options based on protein requirements.

*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.

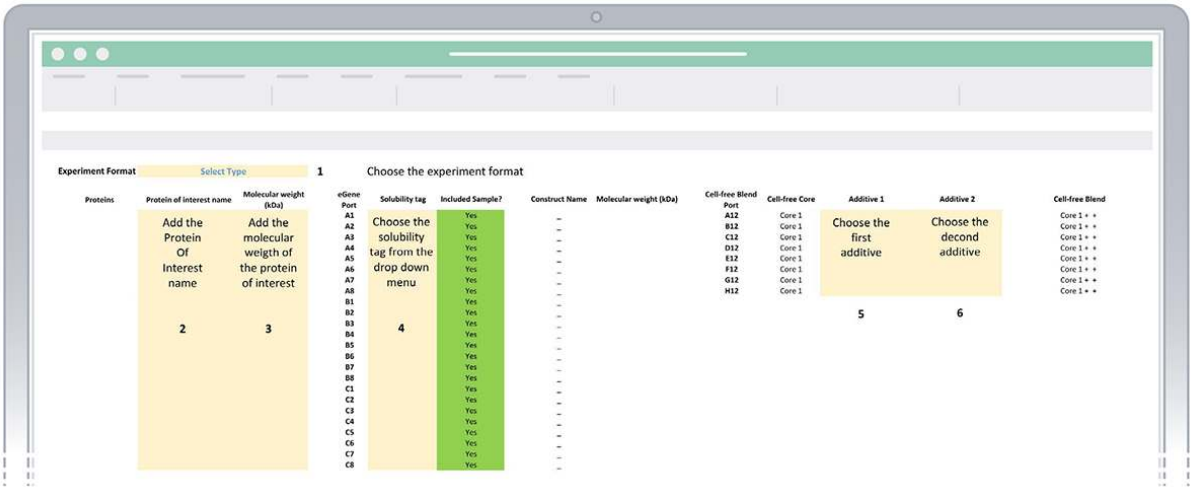
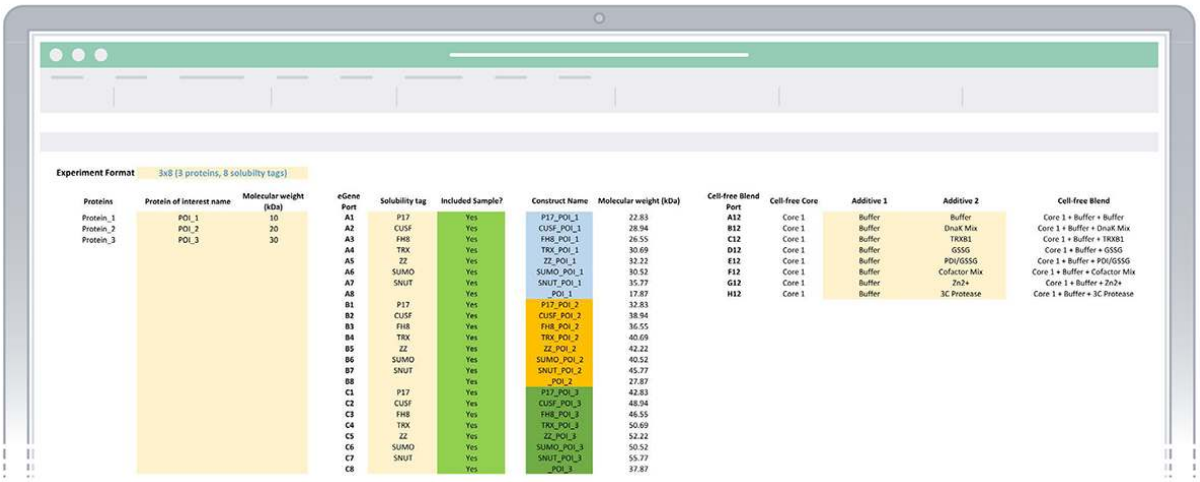


Figure 2: Sheet “1. Enter experiment details” of the eProtein Discovery Standalone template.

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.

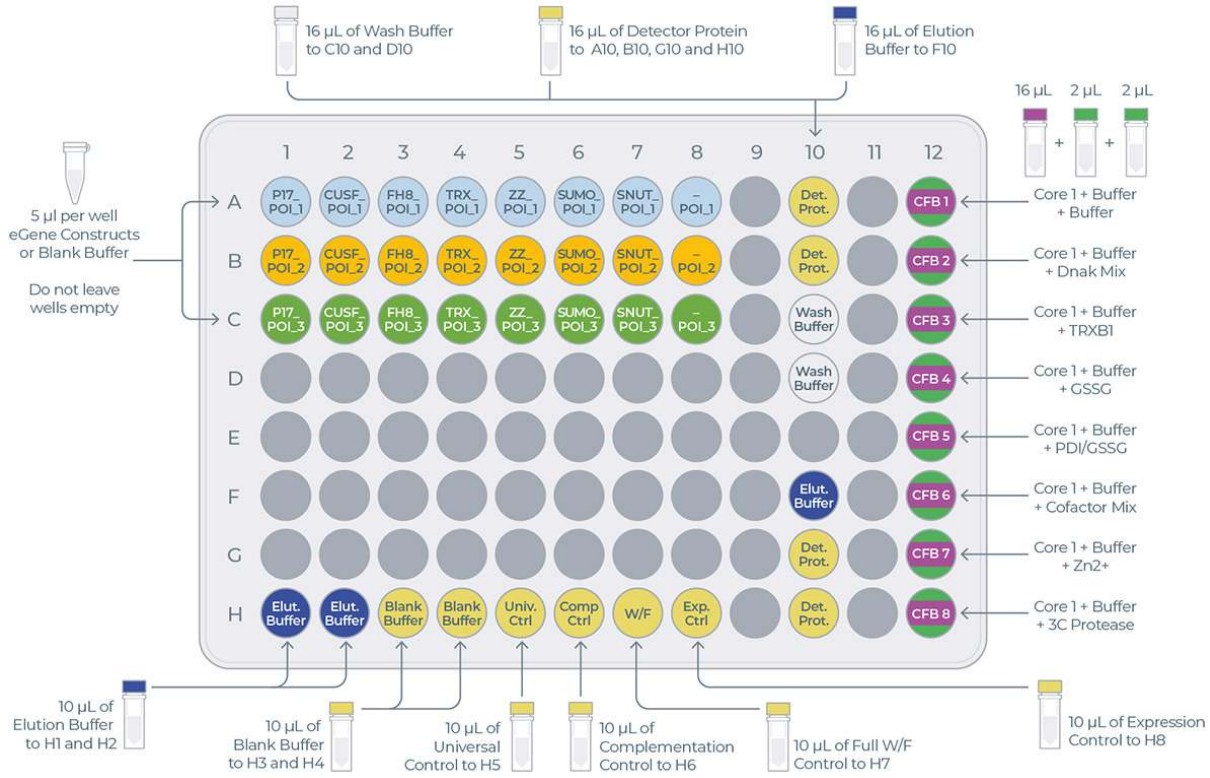


Figure 4: Example of transfer plate design

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).

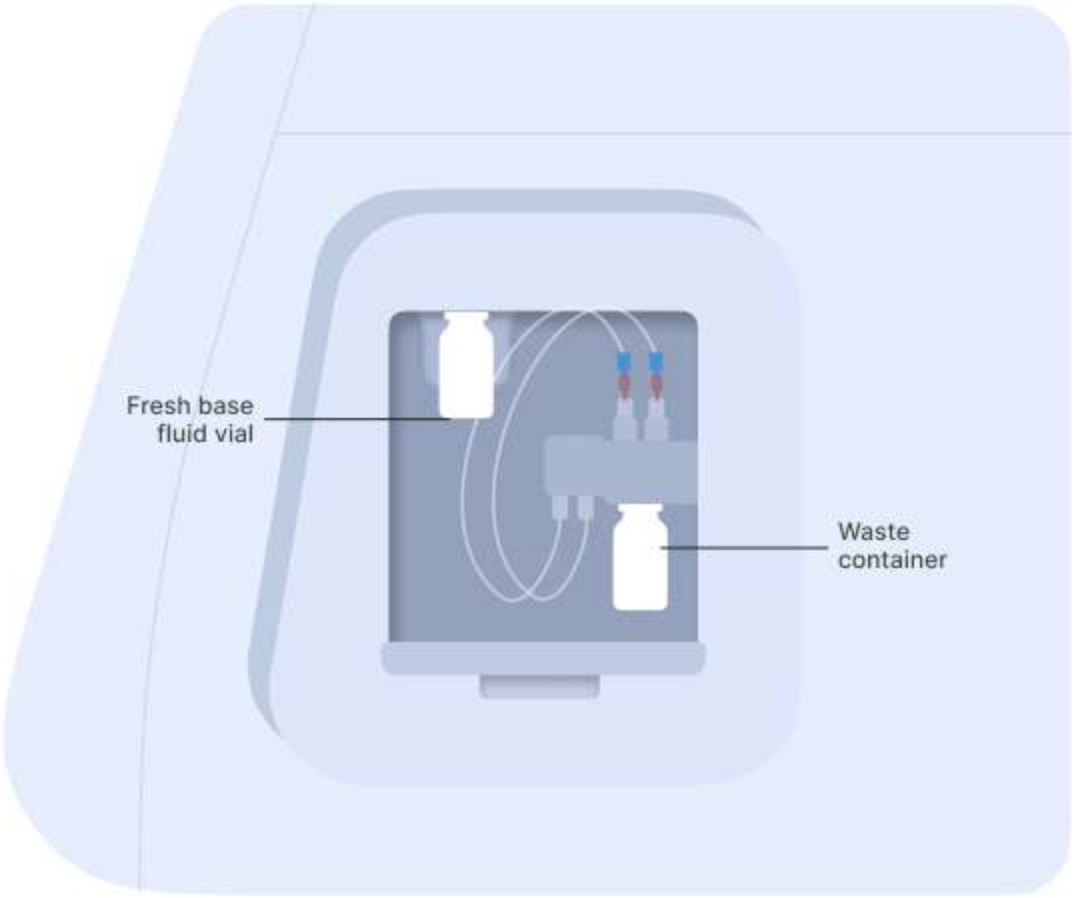


Figure 5: Vial of base fluid and the waste container connected to the pump as shown on the screen

[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.

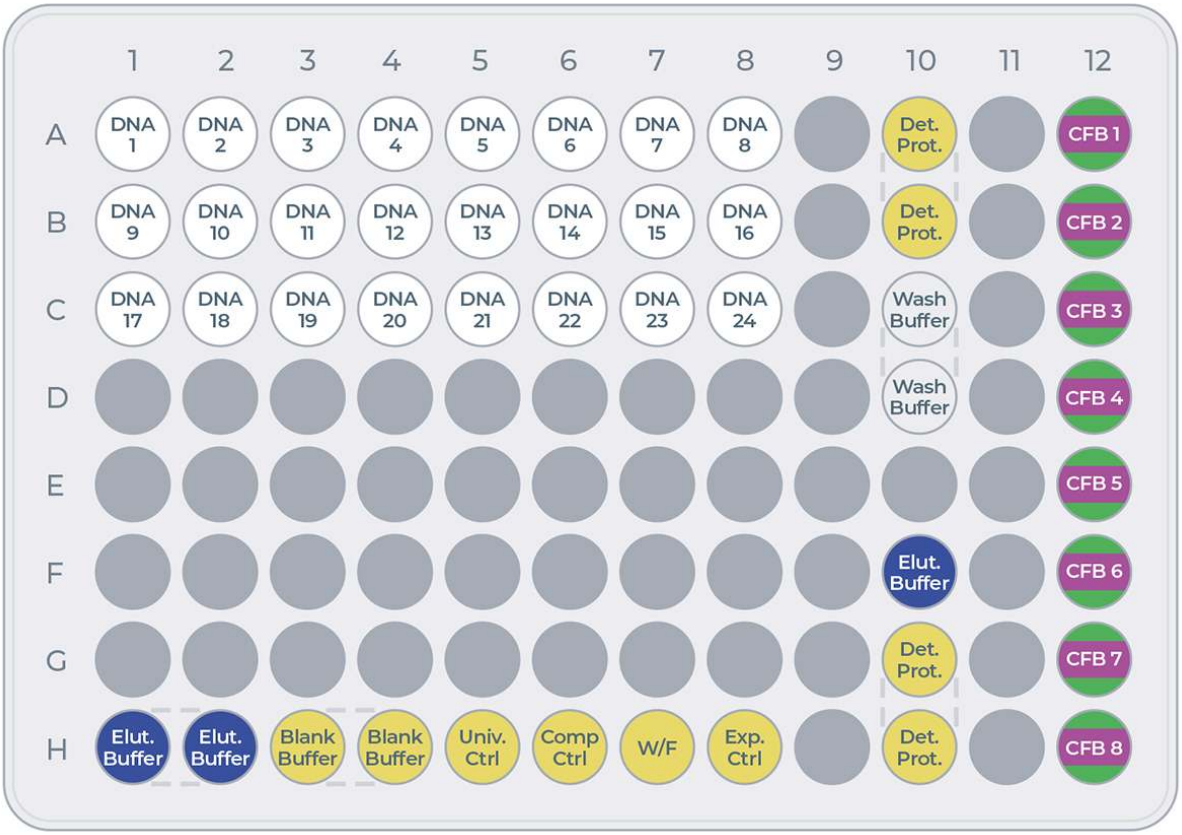


Figure 7: Transfer plate layout

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)

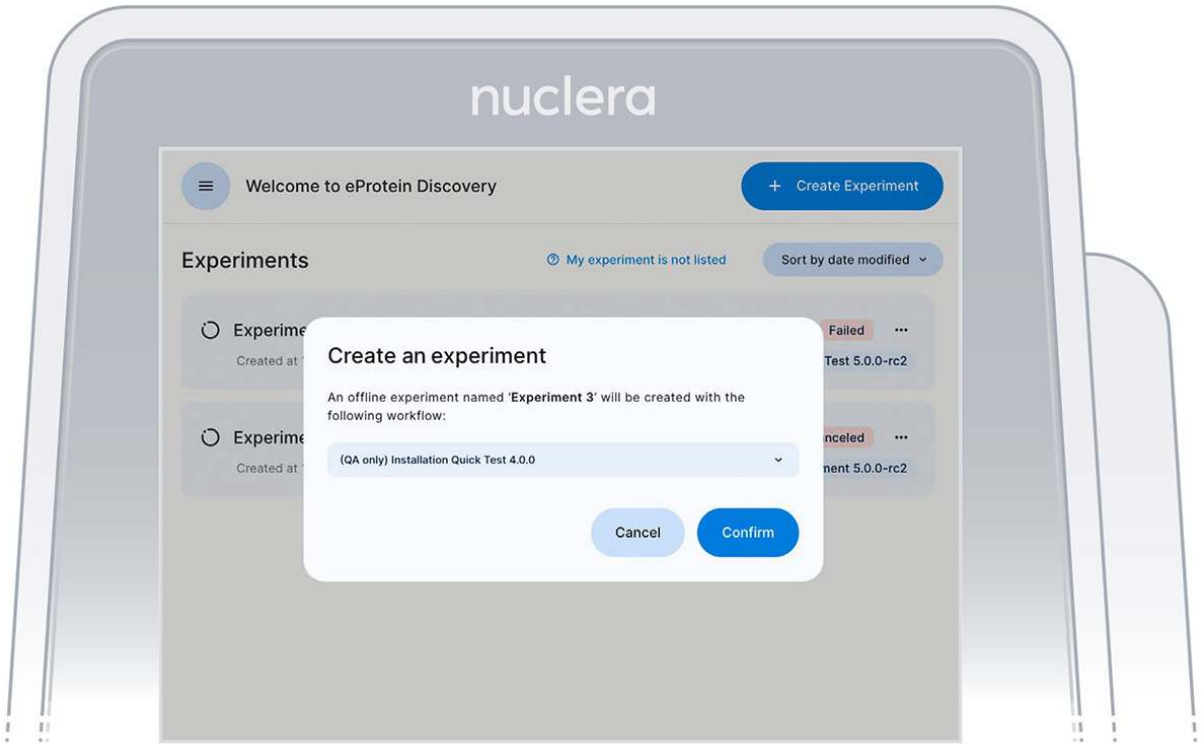


Figure 8: Experimental design screen

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.

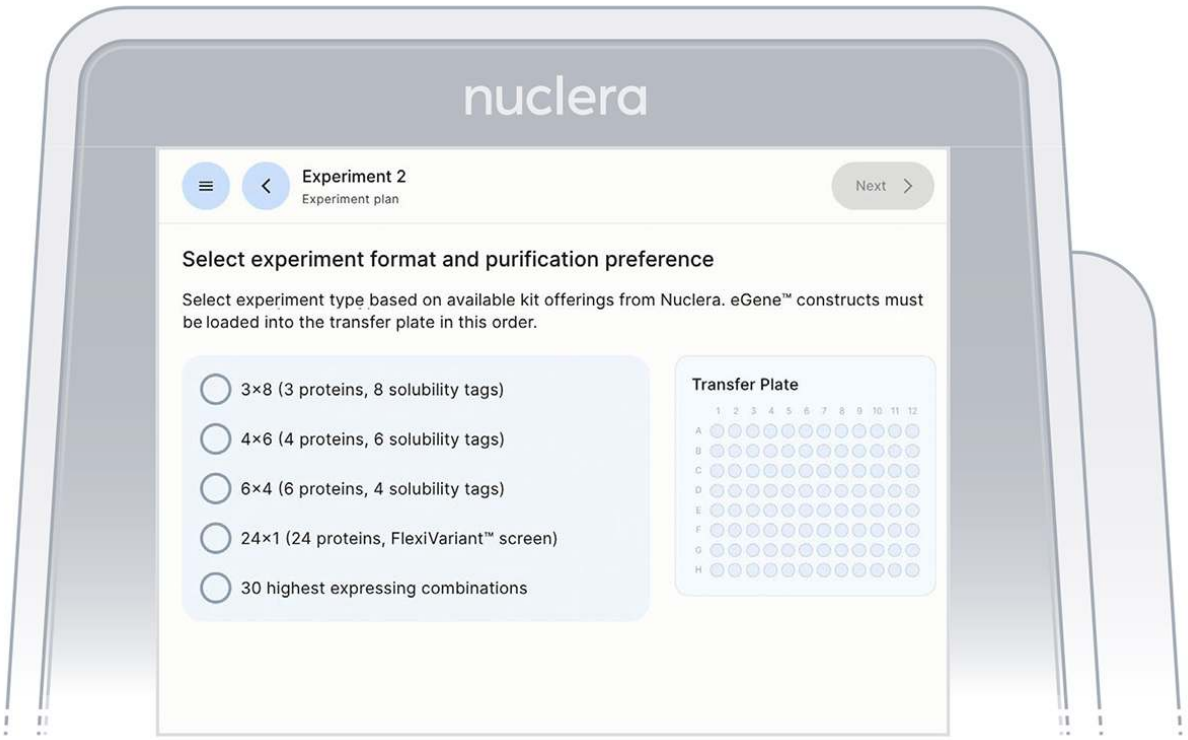


Figure 9: Instrument screen to select the experiment format

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

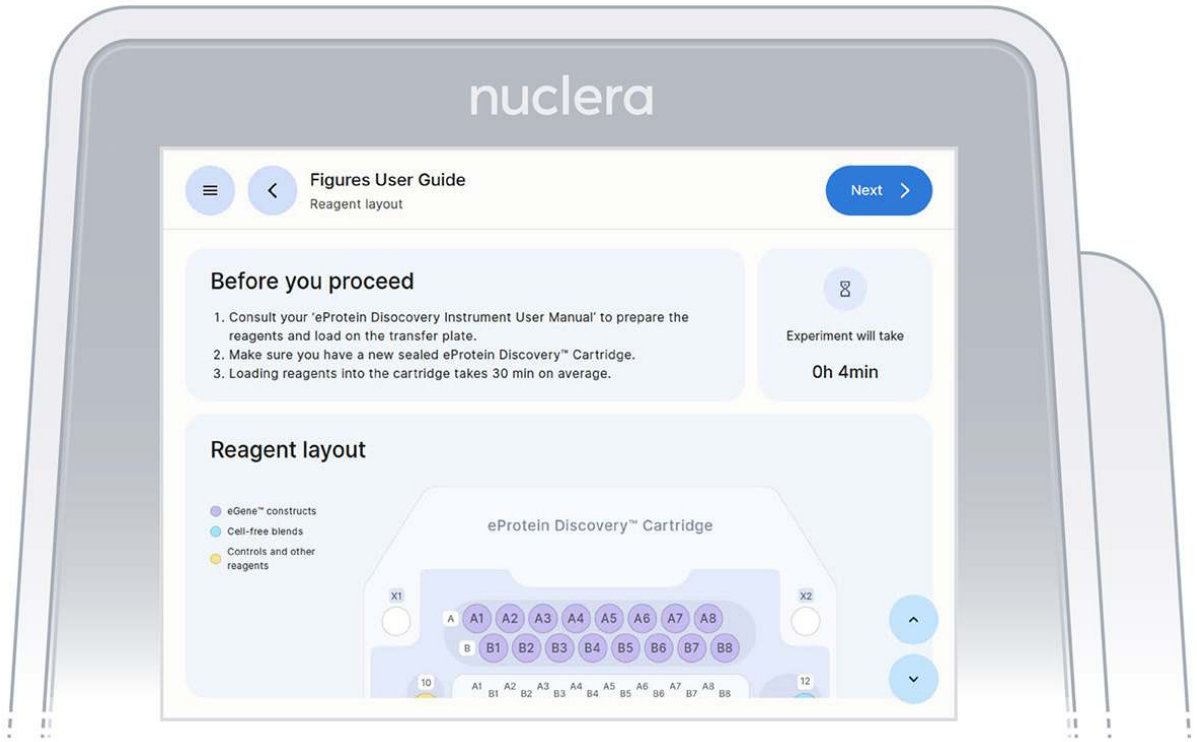


Figure 10: Summary of the selected experiment

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.

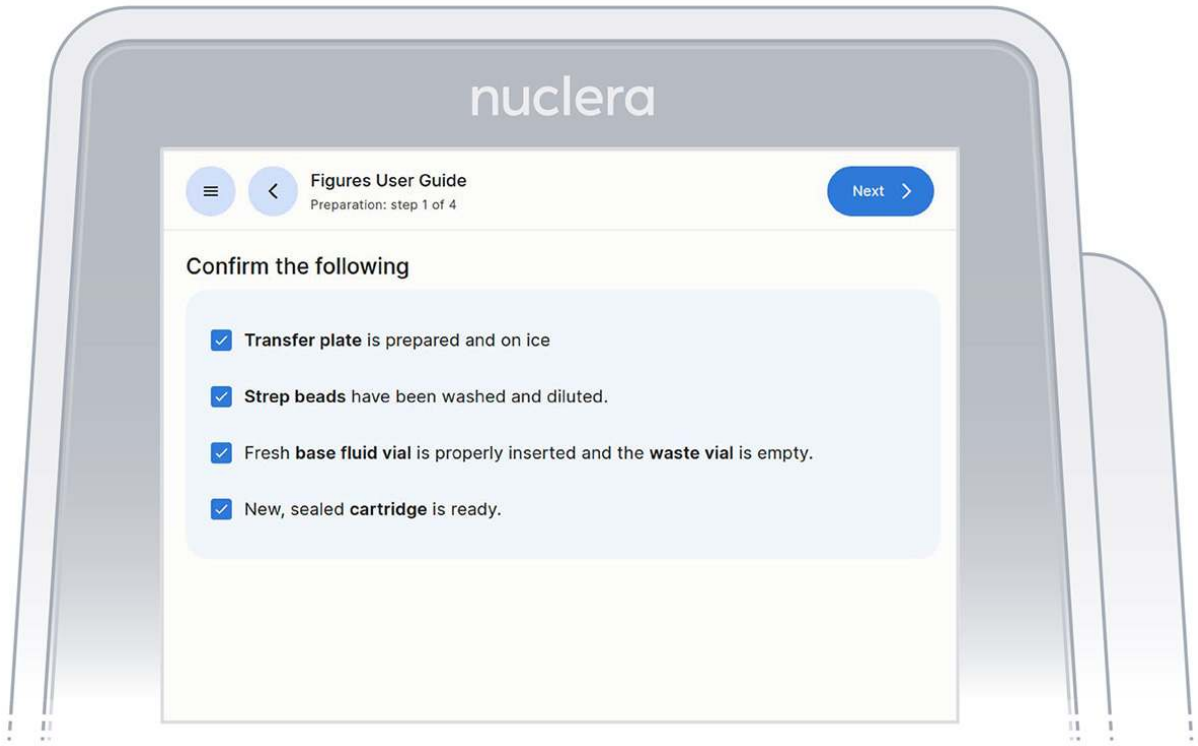


Figure 11: Checklist screen before the experiment starts

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).

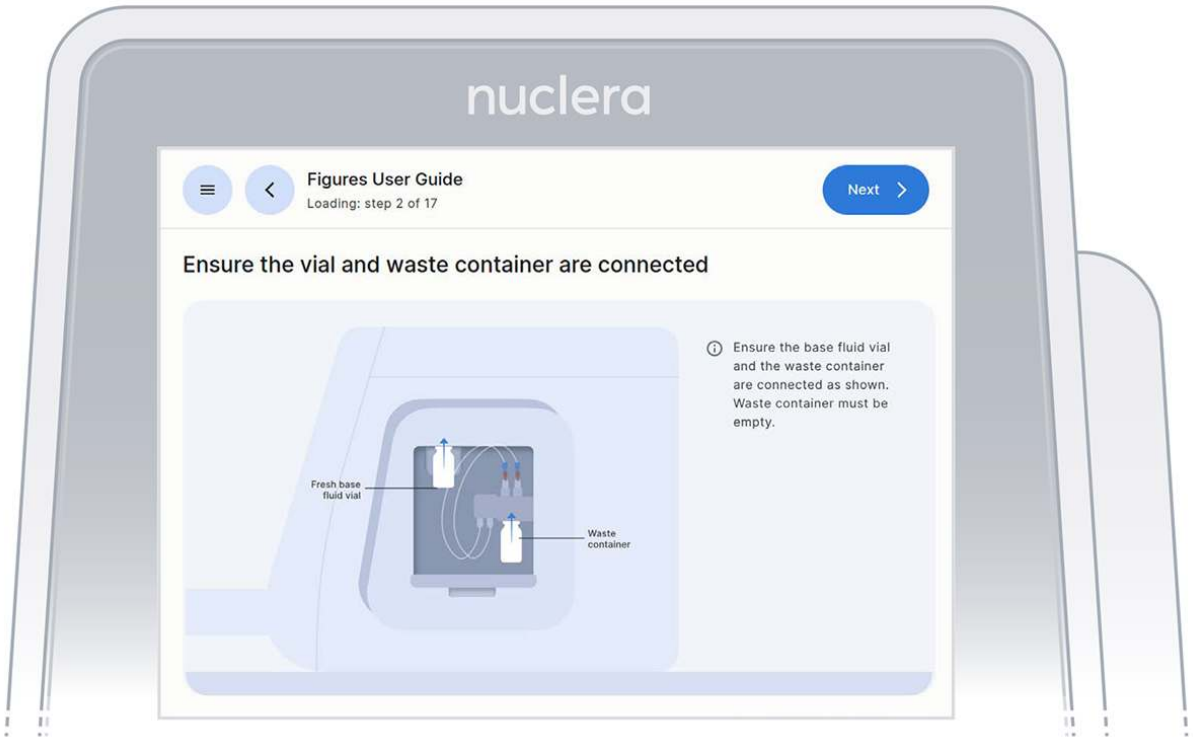


Figure 14: Vial of base fluid and the waste container connected to the pump as shown on the screen

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).

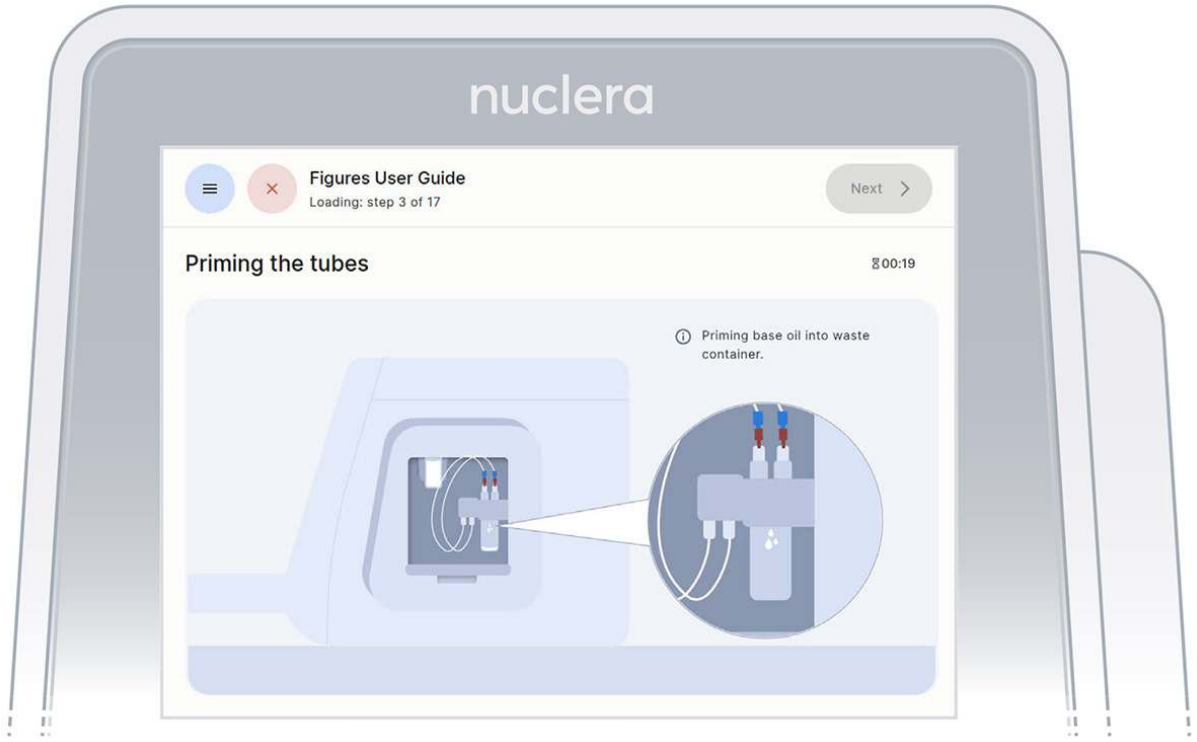


Figure 15: Priming the pump tubings with base fluid

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.

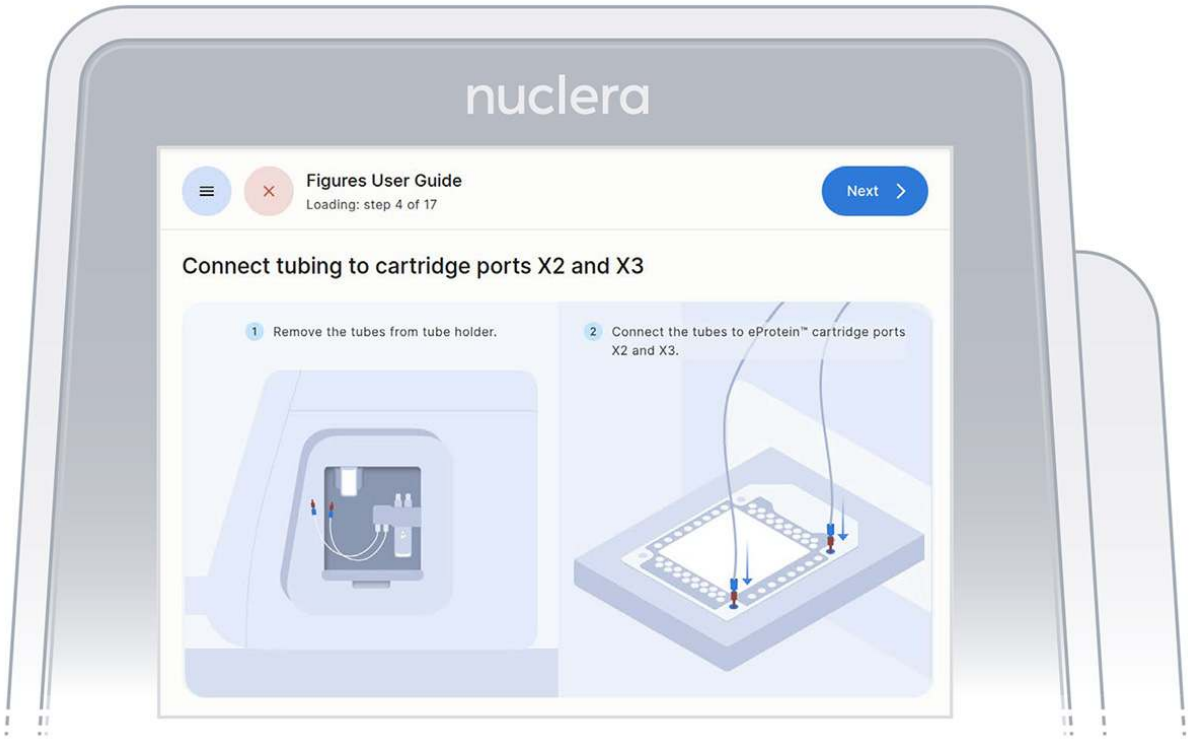


Figure 16: Connection of the pump tubes to the cartridge

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).

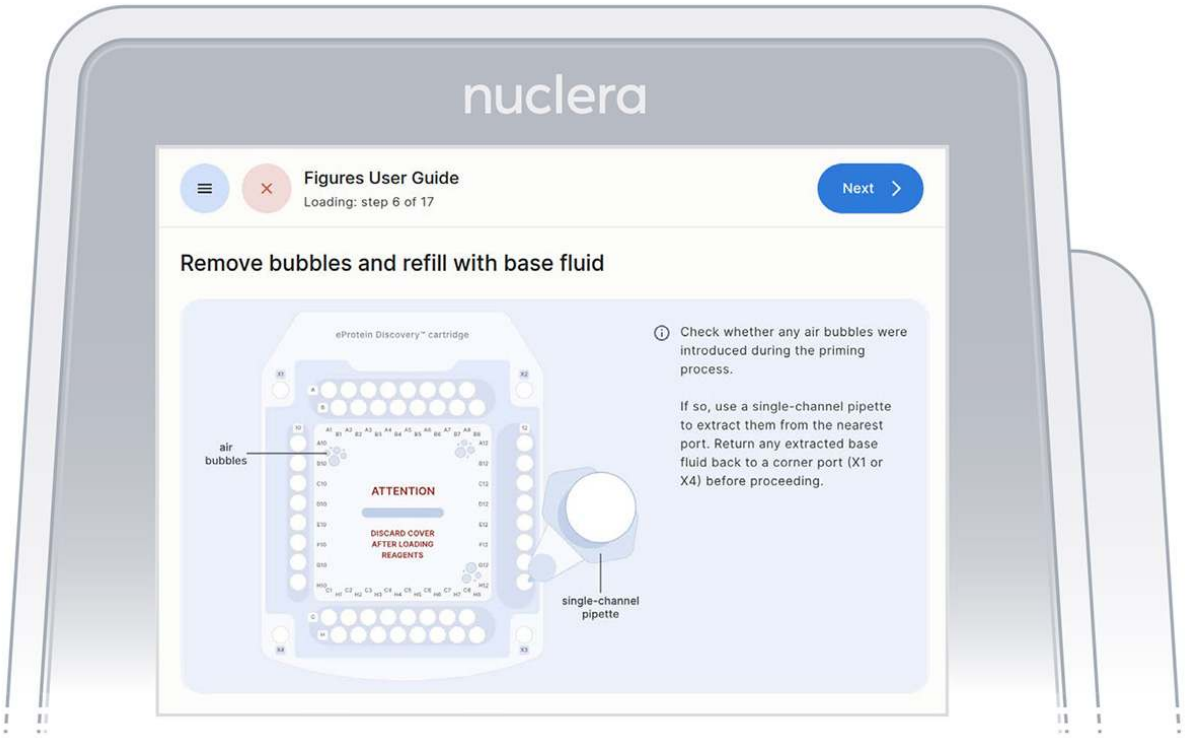


Figure 17: Procedure to remove potential air bubbles from the cartridge

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).

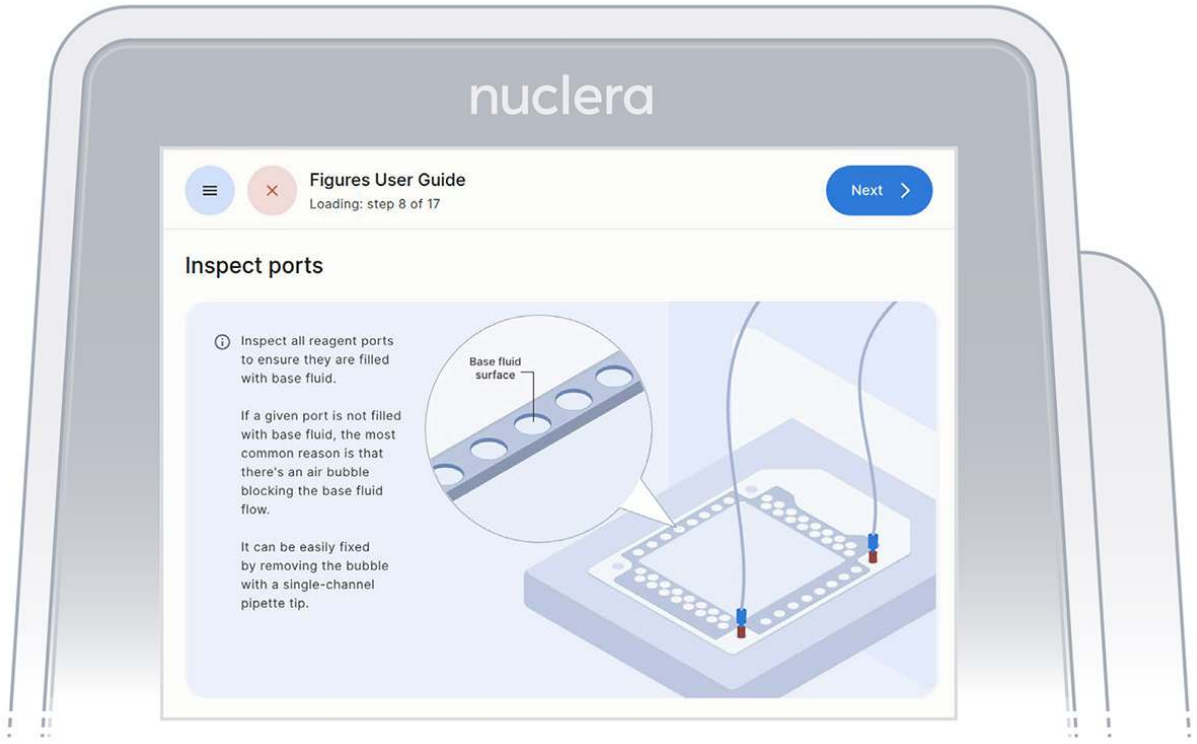


Figure 18: Procedure to remove potential air bubbles from the cartridge

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).

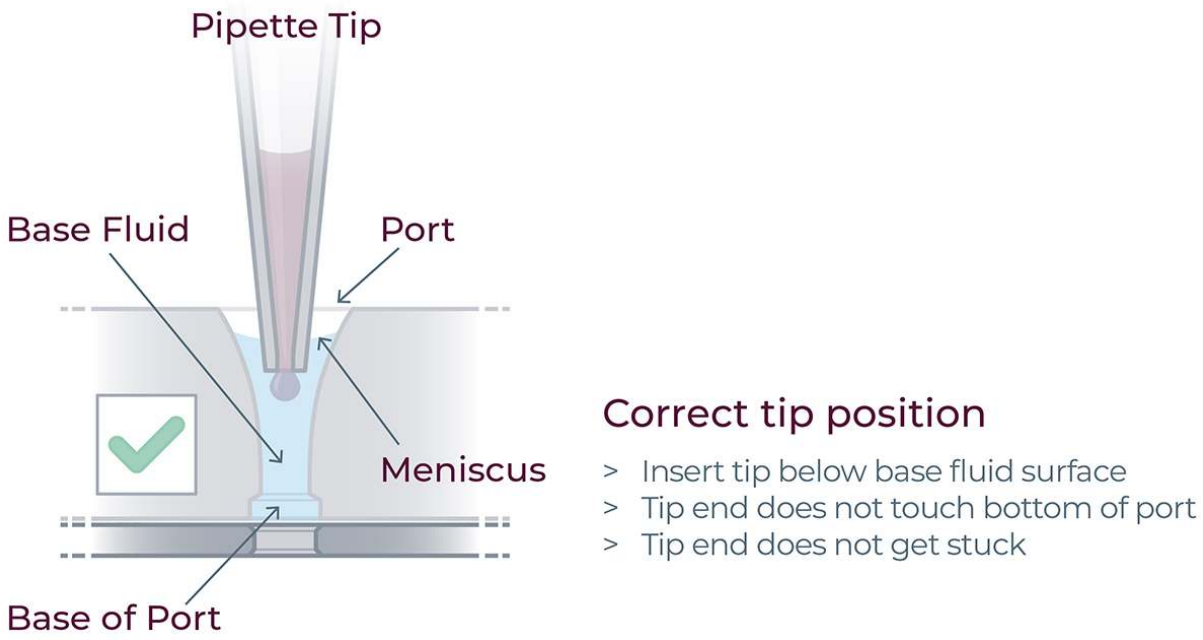


Figure 19: For correct reagent loading the pipette tip is immersed in the base fluid and not touching the bottom of the port

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.

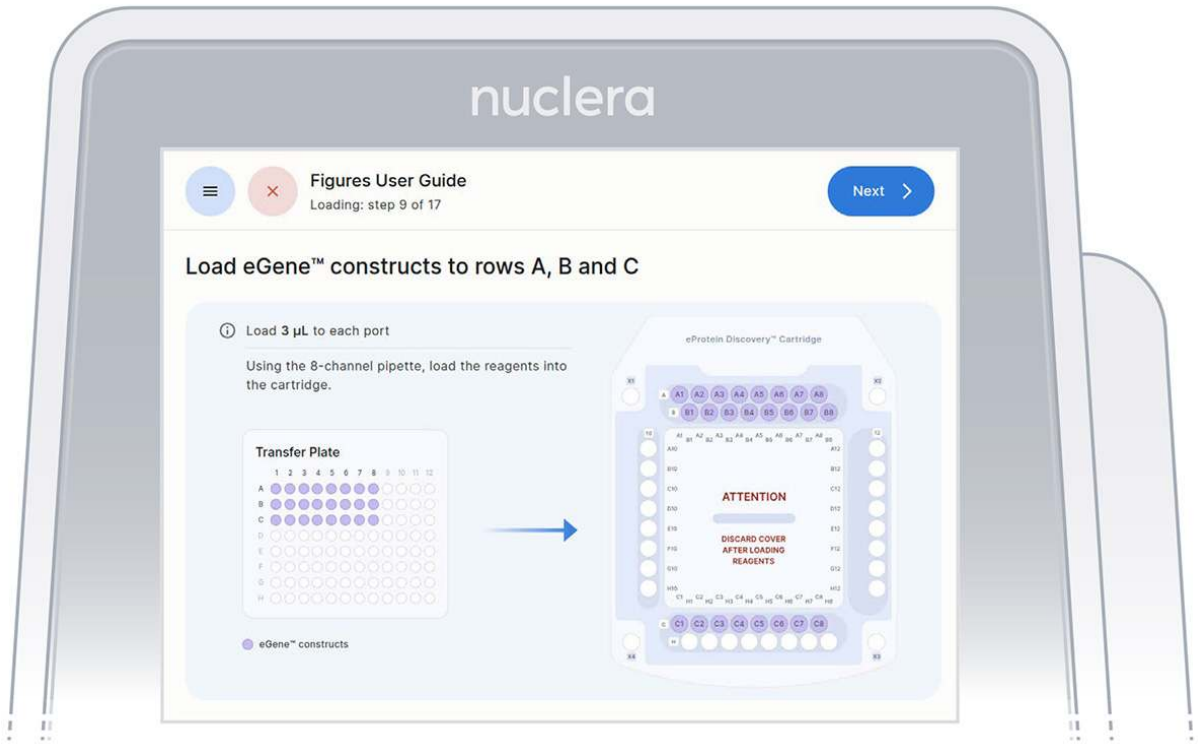


Figure 20: Loading of the eGene constructs onto row A, B and C of the cartridge

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.

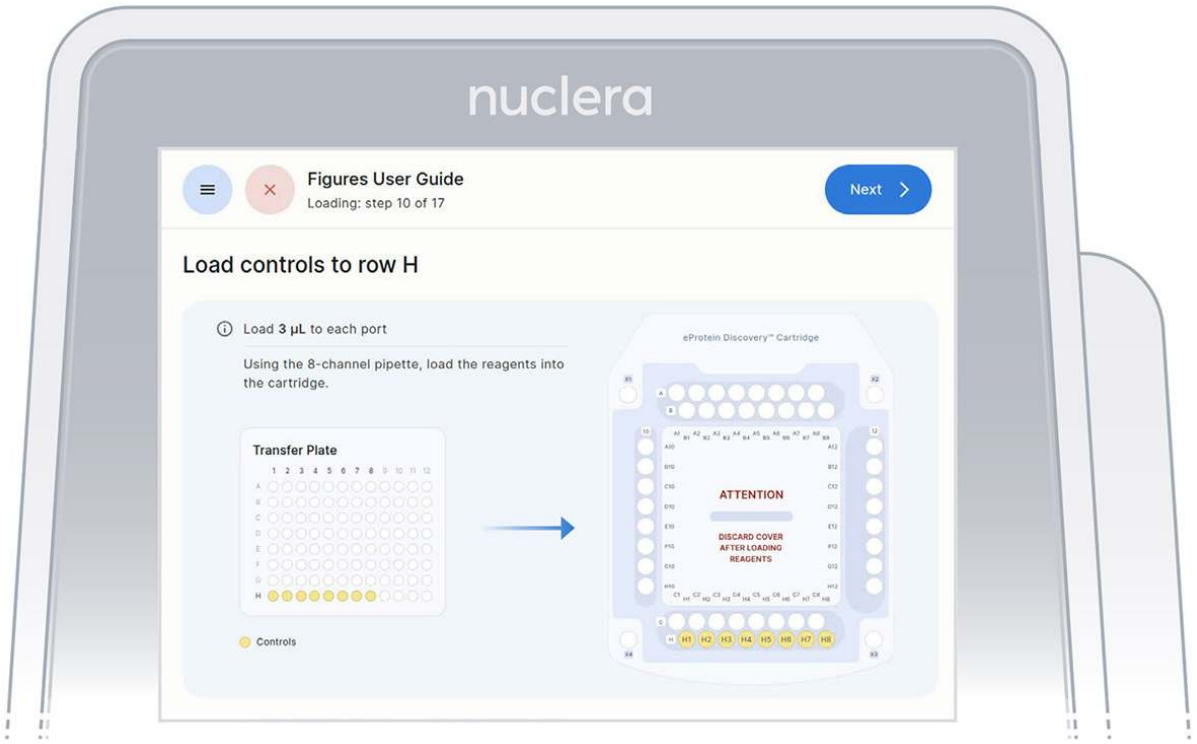


Figure 21: Loading of the reagents onto row H of the cartridge

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).

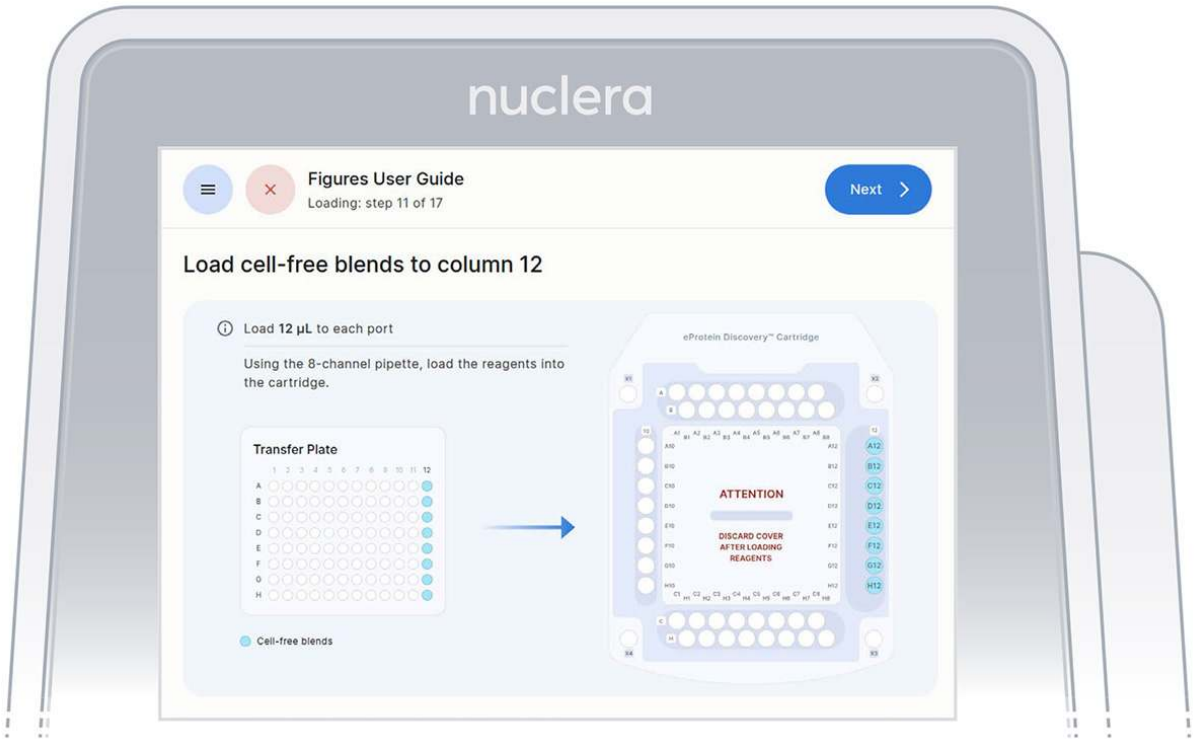


Figure 22: Loading of the Cell-free Blends onto column 12 of the cartridge

▹ 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.

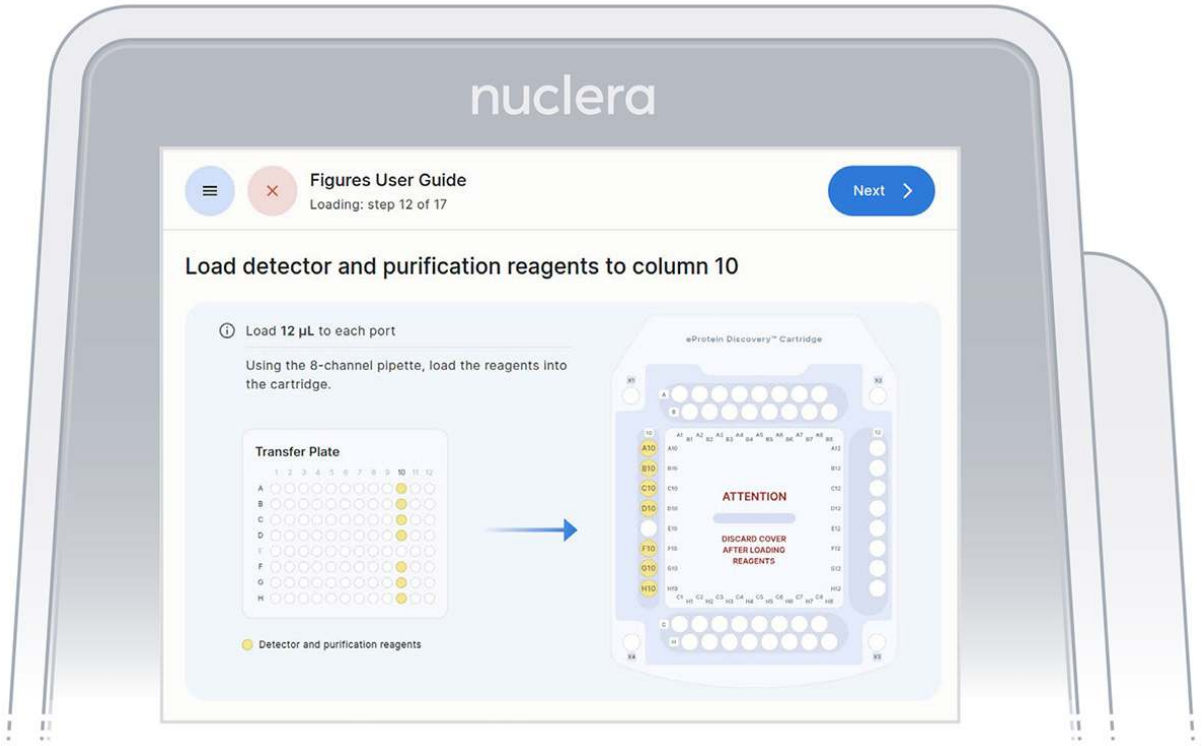


Figure 23: Loading of the reagents onto column 10 of the cartridge

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.

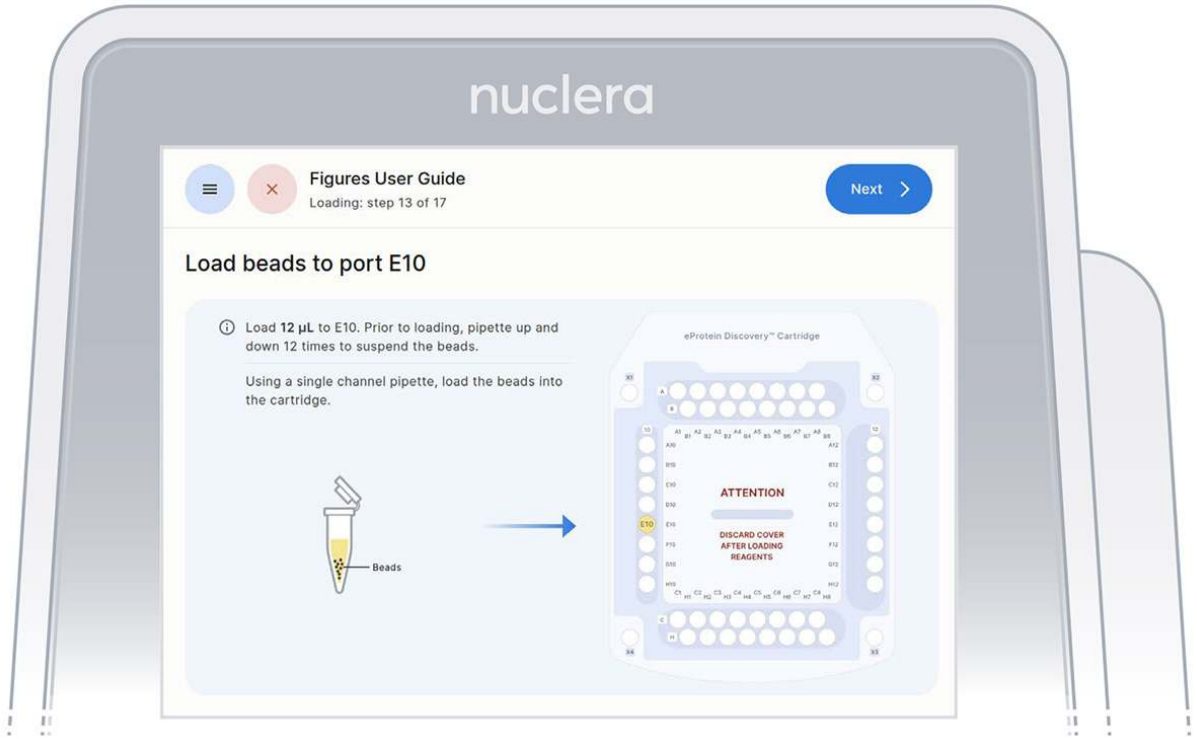


Figure 24: Loading of the Strep beads to port E10 of the cartridge

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

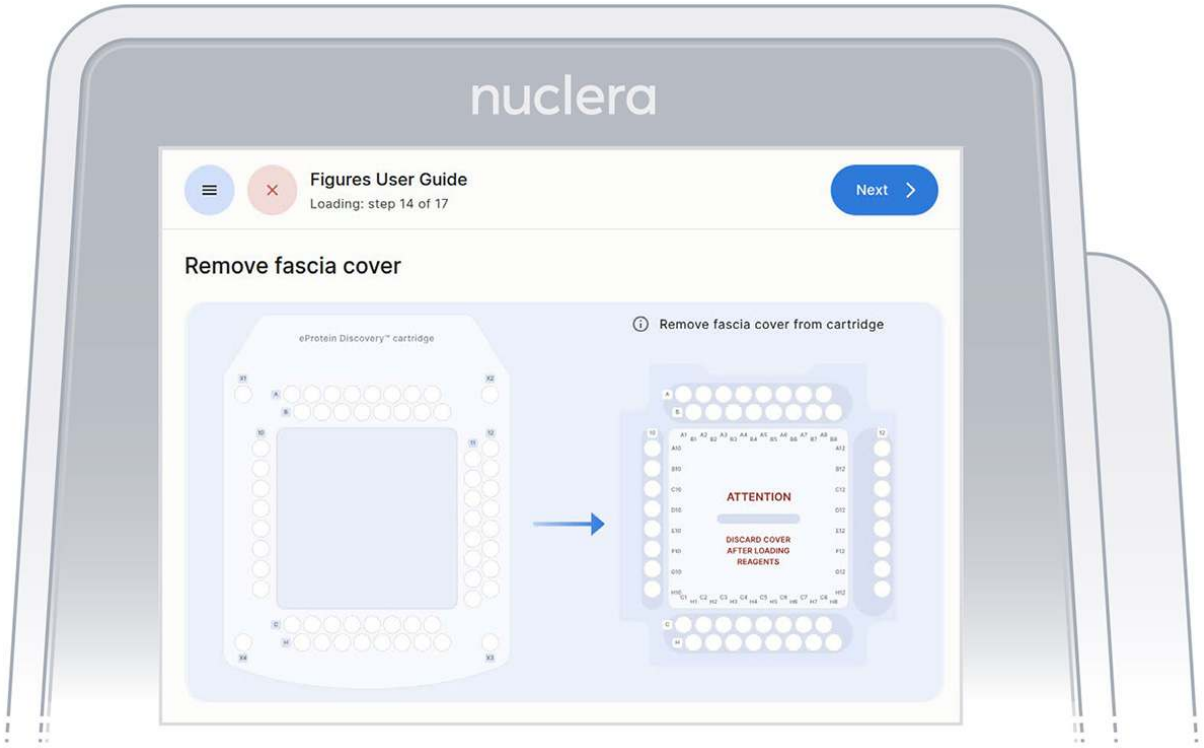


Figure 25: Remove the cover from the cartridge

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).

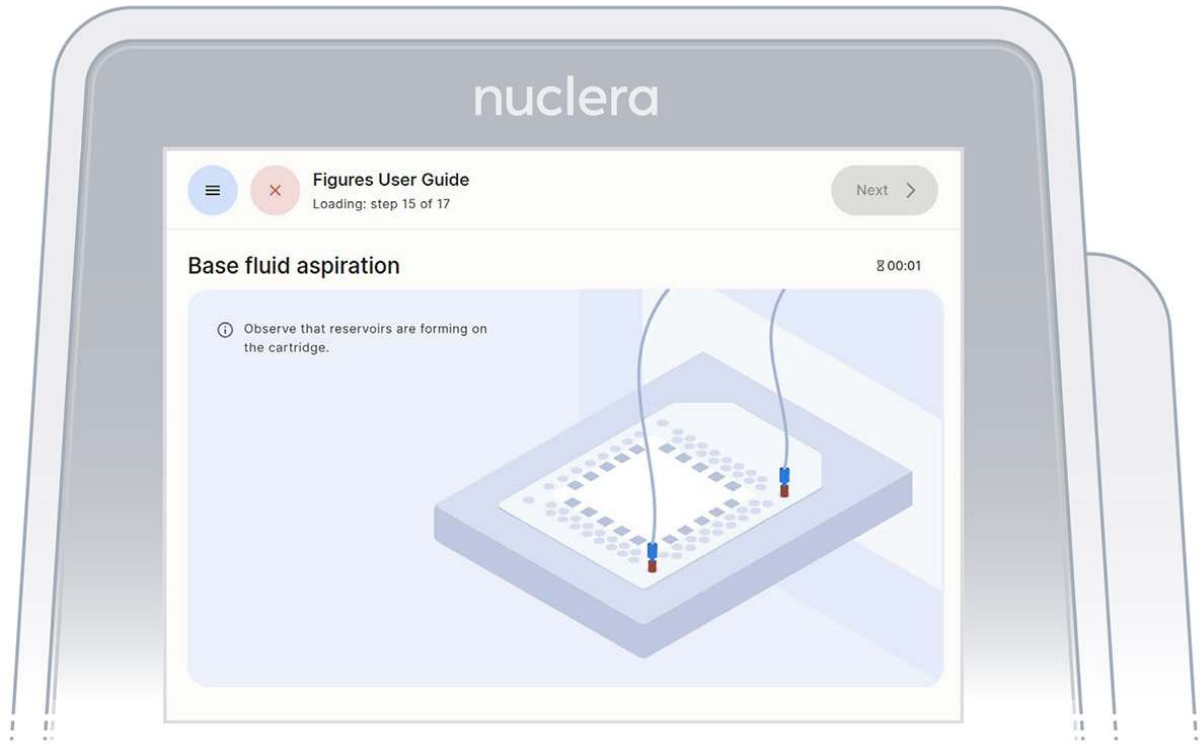


Figure 26: Base fluid aspiration

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.

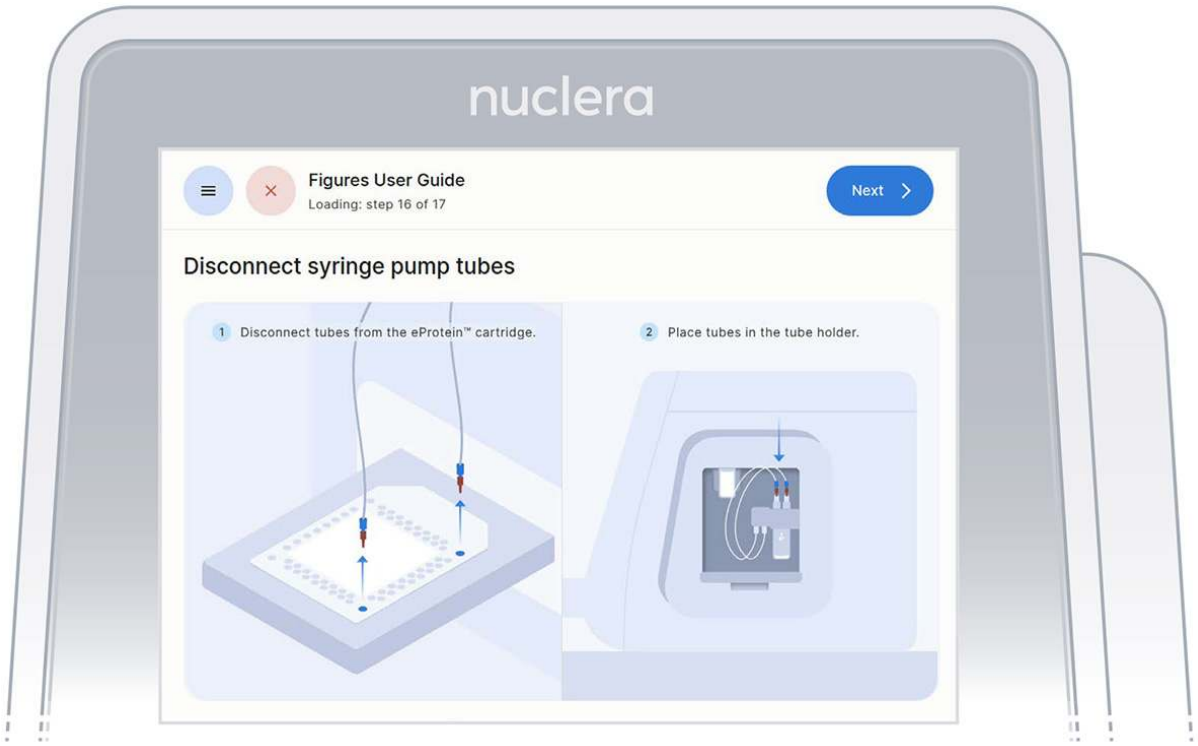


Figure 27: Disconnect the tubes and place them on the tube holder

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

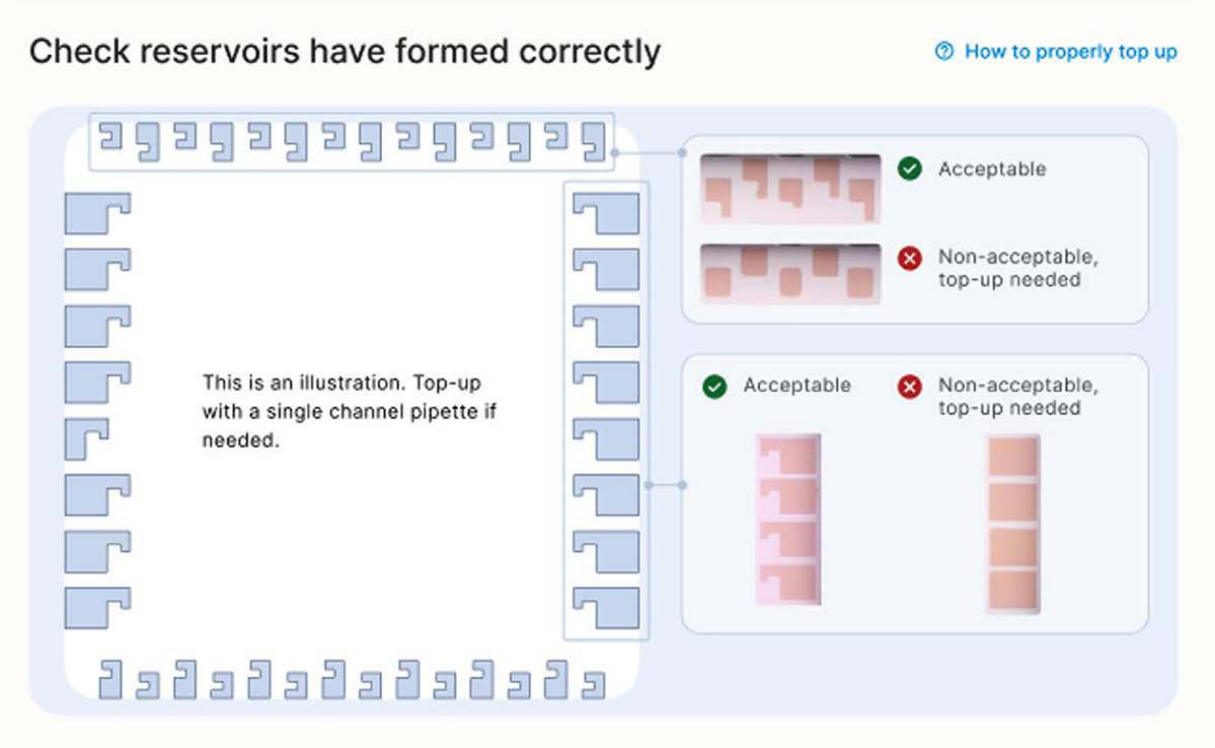


Figure 28: Check the reservoirs have formed correctly on the cartridge

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

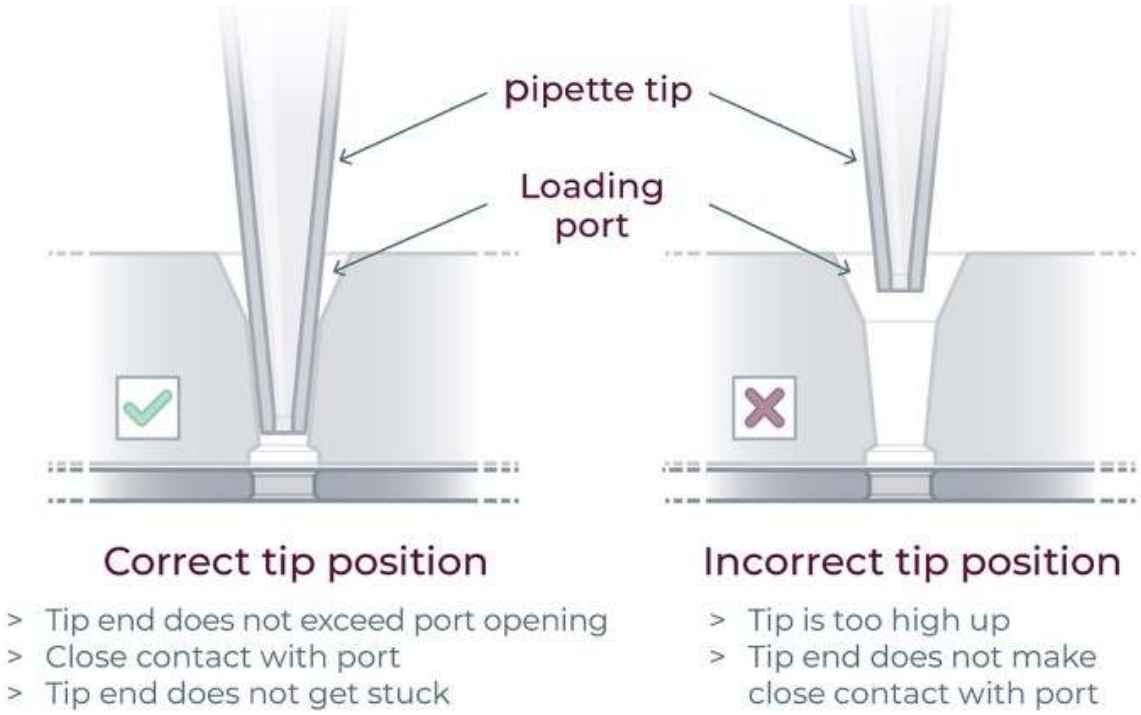


Figure 29: For manual correction of the reagent loading to correctly form the reservoirs on cartridge

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).

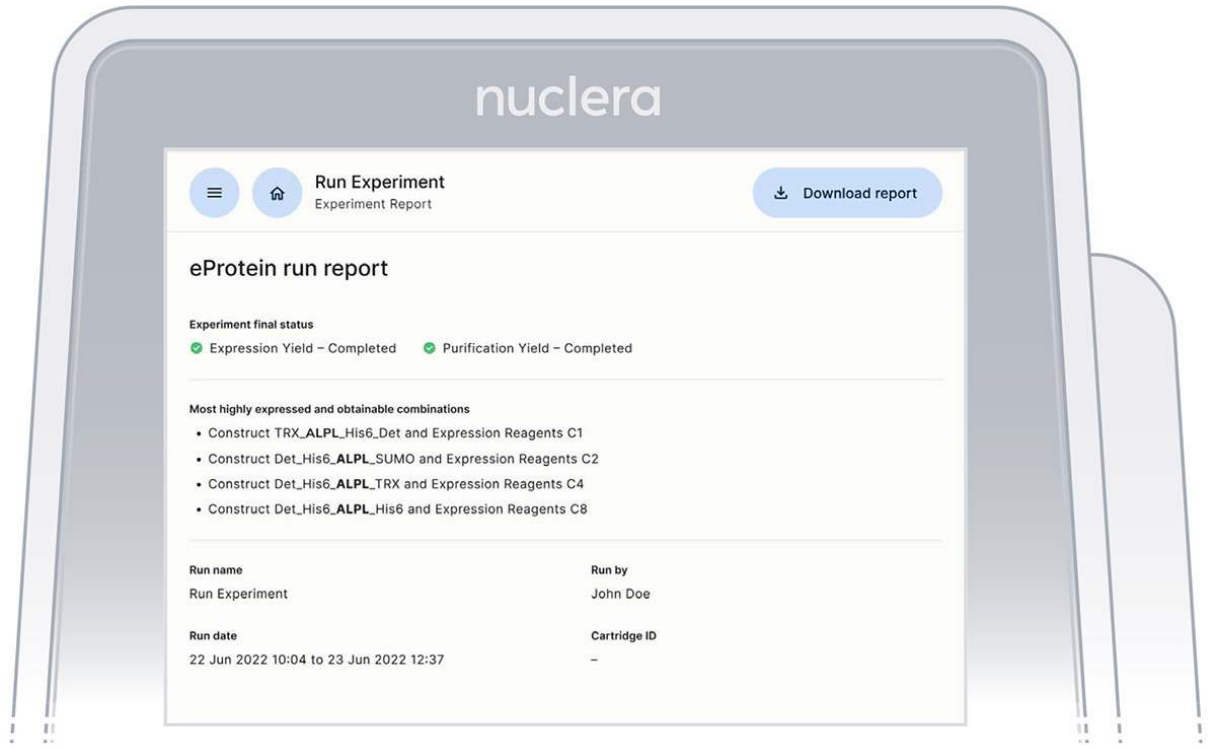


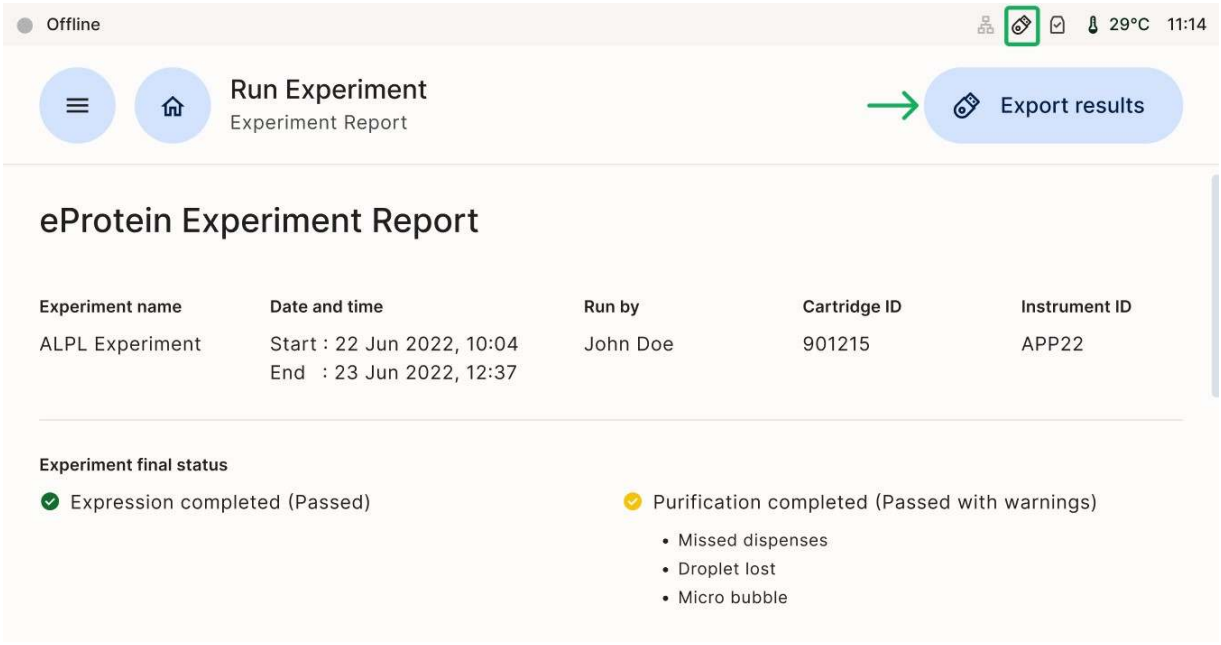
Figure 30: Result screen from the Instrument Software

eProtein Discovery report

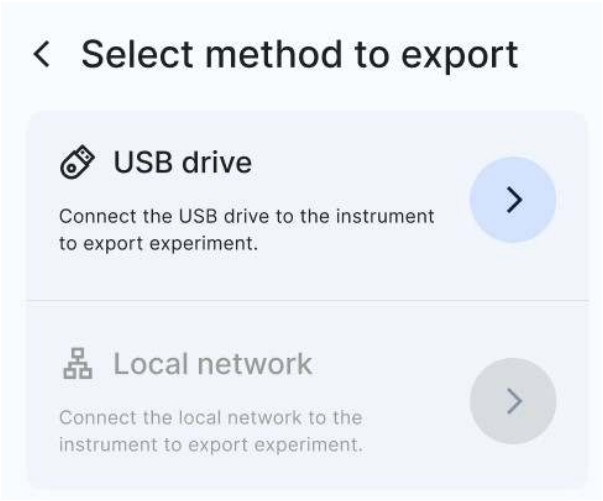
At the end of the experiment a report containing all the information about the experimental setup and the results can be downloaded from the instrument. Data can be exported using a USB, LAN or a Local Laptop. The upload takes about 15 minutes and during this time the [Download Report] button at the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

USB

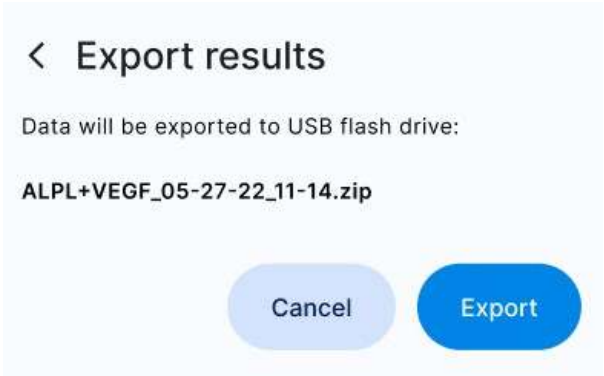
To export data to a USB flash drive it required to have a company-approved USB flash drive for data transfer. Encrypted flash drives are not currently supported. Insert the flash drive on the right hand side of the instrument in the USB port. This will highlight the flashdrive icon on the screen. Click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

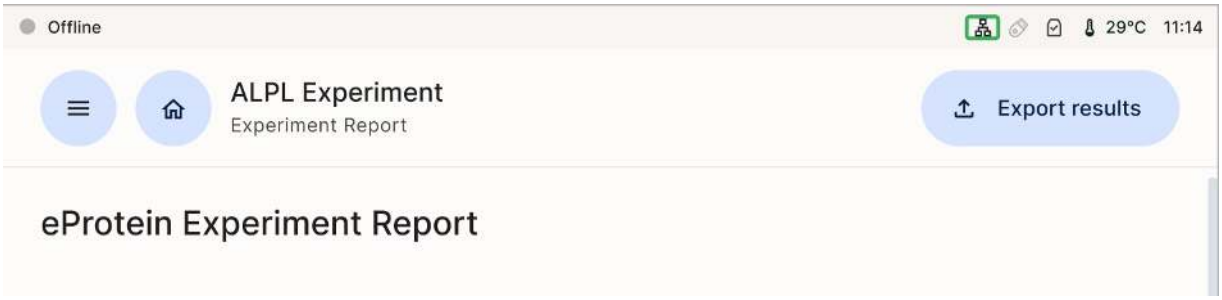


(b)

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

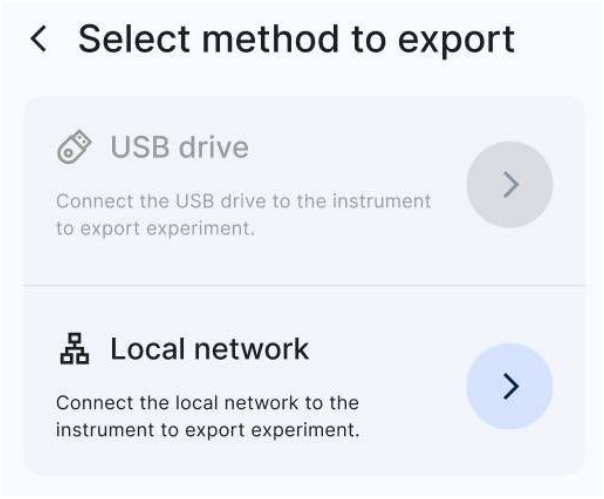
LAN and Local Laptop

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.

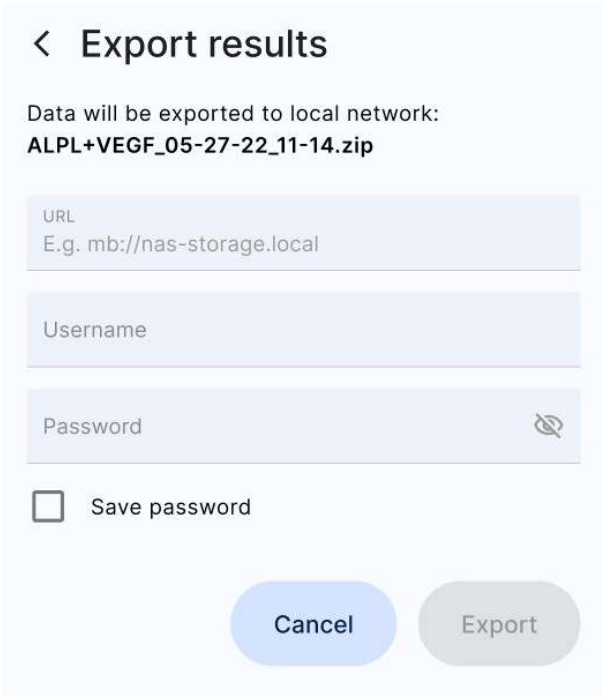


Open a completed experiment and tap the “Export results” button.
Tap (a) "Local network" and enter the following (b):
URL: smb://192.168.1.100/eProteinReports
Username: the name of the Windows user that has access to the shared directory, if this is a domain user then the username should be of the form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

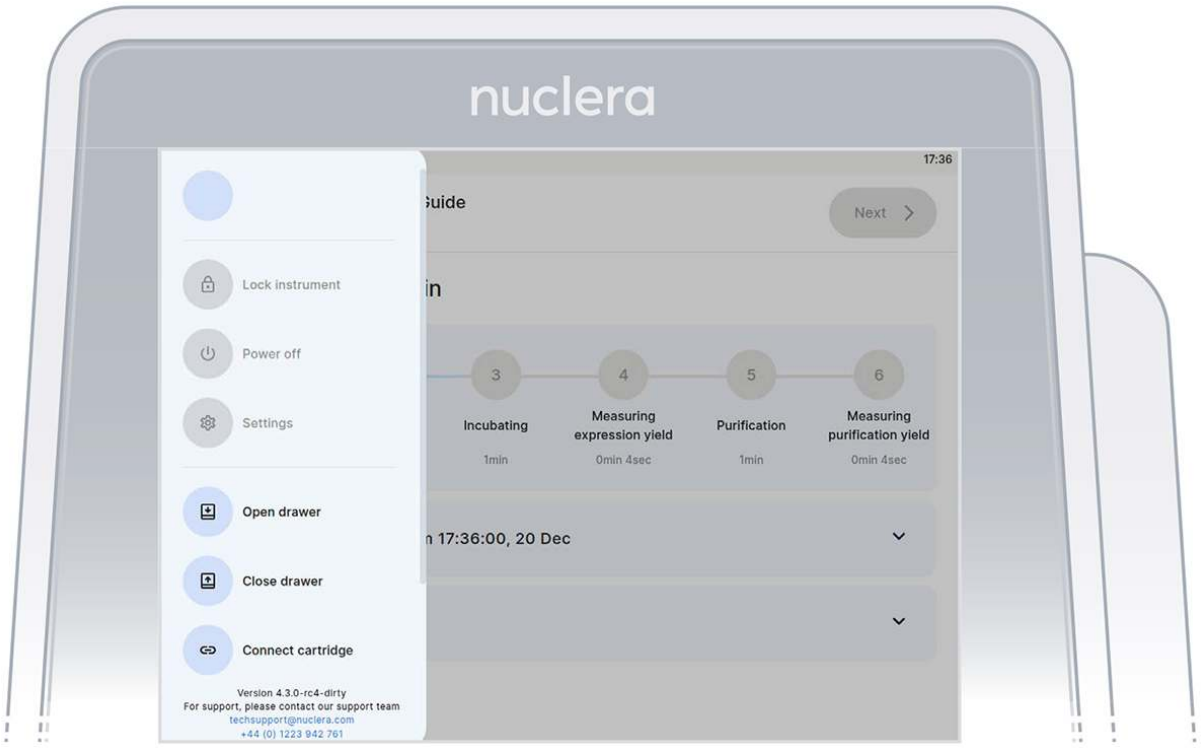


Figure 32: Remove the cartridge from the instrument

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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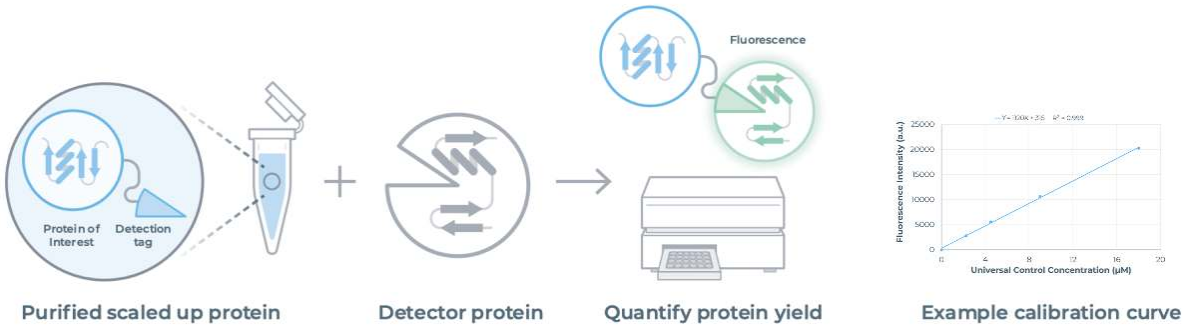
eProtein Quantification Kit Protocol

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).

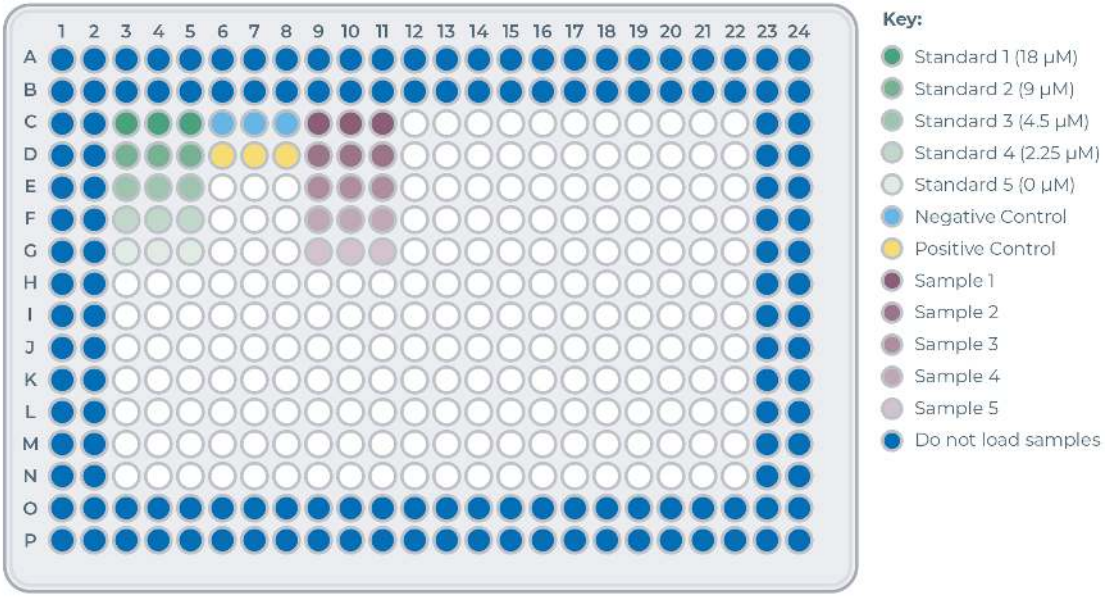
Preparation of Test Samples

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

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📄 How to Connect the Base Fluid Line

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📄 Steps to Remove Bubbles Introduced during Sampl...

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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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