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.

Nuclera Technical Support:

UK Phone / WhatsApp Business: +44 1223 942 761

US Phone: +1 508-306-1297

Email: techsupport@nuclera.com

Offices: Nuclera UK (HQ):

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

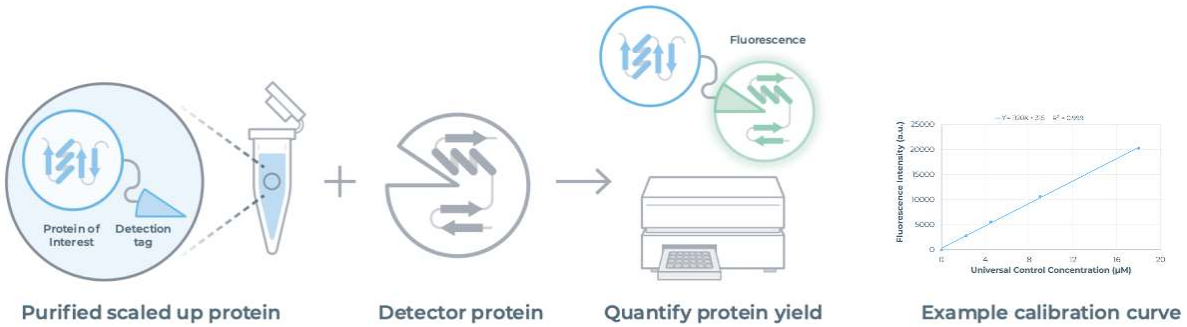
Nuclera USA: 1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

www.nuclera.com

eProtein Quantification Kit Protocol

General Information

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



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

Features and benefits

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

Contents

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

User supplied equipment

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

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

▹ Adhesive PCR plate seal.

Storage and Stability

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

Reaction Assembly Overview

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

Standard Curve Preparation

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

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

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

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

Preparation of Negative Control Reactions

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

Preparation of Positive Control Reactions

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

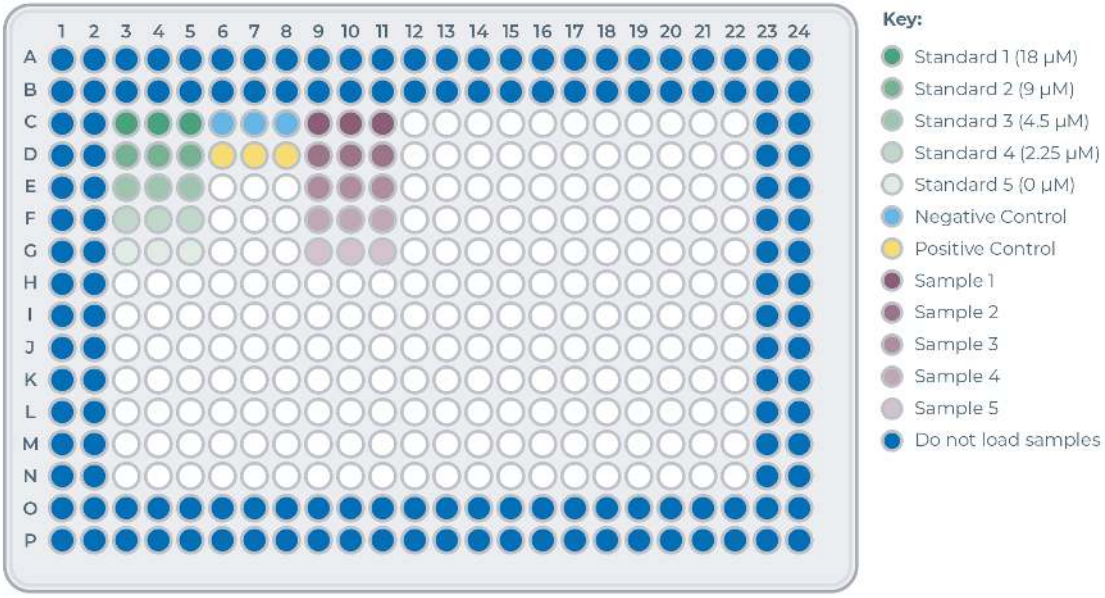
Preparation of Test Samples

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

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

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

Assay Plate Layout Overview



Incubation

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

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

Measurement

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

Data Analysis

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

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

Guidelines to prepare SDS samples

Prepare samples for SDS page gel

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

For the SDS page gel you will need 4 tubes

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

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

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

Load the SDS PAGE gel

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

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

Run at 200 V for 40 minutes

Protein Concentration & Buffer Exchange Guidelines

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

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

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

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

✅ Recommended Practice

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

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

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

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

🚫 Avoid

- Do not use concentrators with < 30 kDa MWCO when using buffers

from the scale-up kit, as they will likely retain and concentrate detergents. This could cause problems with solution phase separation, and impact the quality of recovered concentrated protein.

Videos

See our range of videos

AlphaFold

Seamless integration of AlphaFold into Nuclera's eProtein Discovery™ Cloud softwa...

How to Set Up a Run

Part of the in-the-lab instructional video series

How to Carry Out a Band-Stab Procedure

Part of the in-the-lab instructional video series

Best Practices for Sample Loading

Part of the in-the-lab instructional video series

📄 How to Connect the Base Fluid Line

Part of the in-the-lab instructional video series

📄 Steps to Remove Bubbles Introduced during Sampl...

Part of the in-the-lab instructional video series

AlphaFold

Seamless integration of AlphaFold into Nuclera's eProtein Discovery™ Cloud software, enabling a powerful, structure-guided approach to protein engineering.

Researchers can now go from sequence to purified, soluble protein in just 48 hours by combining AI-driven 3D structure prediction with automated screening. The intuitive interface allows users to visualize protein structures, analyze critical features like domain annotations, hydrophobicity, and residue-level confidence, and strategically design protein variants that balance function with manufacturability.

Key capabilities featured in this demonstration include:

- Interactive 3D modeling of predicted protein structures with zoom, rotate, and residue-level insights.
- Dynamic visualization modes to assess structural confidence, functional domains, and hydrophobic regions.
- Integrated protein variant editor to facilitate rational design and truncation/mutation decisions.
- Bioinformatic validation tools such as multiple sequence alignment, pLDDT, and PAE plots to guide expression optimization.

Through AlphaFold integration, protein design becomes a data-informed, high-efficiency workflow — empowering researchers to solve protein folding and solubility challenges with unprecedented speed and precision.

Loading...

How to Set Up a Run

Part of the in-the-lab instructional video series

Loading...

How to Carry Out a Band-Stab Procedure

Part of the in-the-lab instructional video series

Loading...

Best Practices for Sample Loading

Part of the in-the-lab instructional video series

Loading...

How to Connect the Base Fluid Line

Part of the in-the-lab instructional video series

Loading...

Steps to Remove Bubbles Introduced during Sample Loading

Part of the in-the-lab instructional video series

Loading...