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The eProtein Discovery™ System

Rapid protein access at your fingertips

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The eProtein Discovery™ system

The current conventional method for obtaining protein is laborious and fragmented, requiring a combination of multiple workflows such as cloning, sequencing, transformation, expression, and purification. Often, many rounds of construct iteration and expression tests are required when the first few constructs fail to produce active, soluble protein, leading to prolong discovery timeline and frustration. It can take weeks to months to obtain enough soluble protein for downstream applications.

Using Nuclera's eProtein Discovery system, scientists can automate multiple construct expression and purification screens in parallel to identify the best conditions for obtaining soluble proteins. One can then scale up protein expression the next day, to obtain micrograms of protein in-hand, in less than 48 hours; sufficient to power biochemical and biophysical assays.

Nuclera's technology integrates cell-free protein synthesis (CFPS) and digital microfluidics on eProtein Discovery™ Cartridges to enable screening of 192 expression conditions in one run. The rapid workflow informs users which construct works best and sheds light on any necessary additives to promote proper protein folding. The eProtein Discovery system offers the power of knowledge to users to make protein decisions quicker, shortens lead-time to protein and facilitates progress in drug discovery and research.

eProtein Discovery protein expression and purification system provides users with the following benefits:

- Rapid, automated expression and purification evaluation of 24 construct variants and screening against 8 different expression blends
- Flexibility in experimental design planning at your desktop/laptop
- Validated reagents to ensure success for on-cartridge screen and off-cartridge scale up
- Small footprint to fit into the tightest lab space
- Intuitive touch screen with easy to follow step-by-step guide
- Reliable system with components integrated for complete automated workflow on the instrument

System Overview

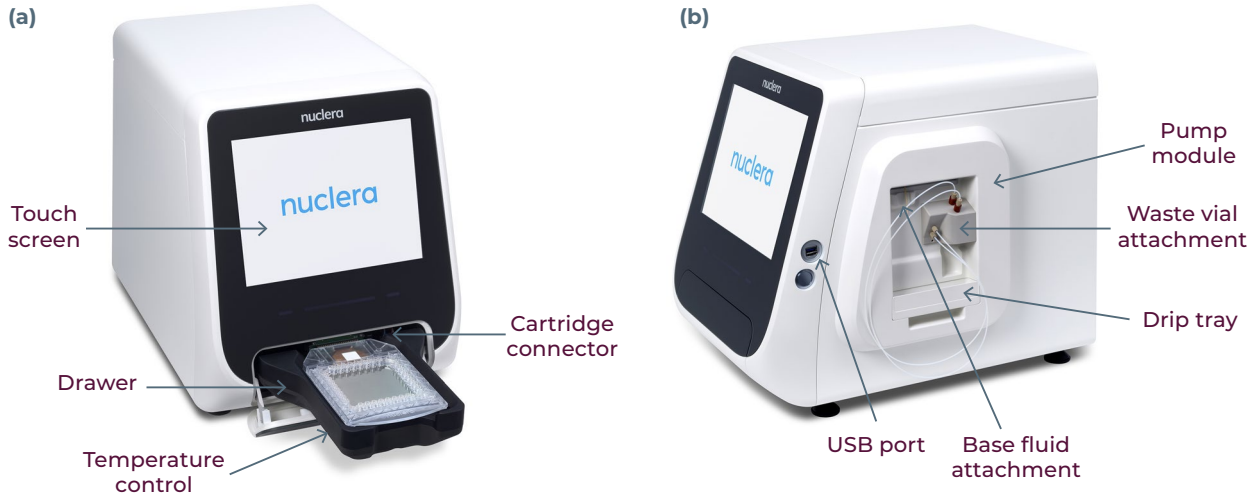


Figure 1. (a) Front and (b) side view of the eProtein Discovery Instrument depicting the various components and features.

eProtein Discovery™ Instrument

This small and lightweight instrument accelerates protein expression and purification pilot studies conveniently at the user's benchtop, replacing the need for costly liquid handlers and various small-scale expression and purification instruments. Designed for all levels of scientists, the instrument provides an easy-to-learn, step-by-step guide to streamline the workflow from DNA to protein. Integrated features include:

- **Motorized drawer**- Opens and closes cartridge holding platform controlled on the touch screen. Secures the cartridge in position and connects the cartridge to the instrument. Error proof cartridge loading design enables cartridges to be positioned on the drawer accurately each time
- **Temperature control** - Steadily maintains the cartridge at the ideal temperature for protein expression and purification regardless of environmental temperature
- **Integrated touchscreen** - Simplifies work environment and reduce bench clutter
- **Base fluid priming station** -Integrated pump system automates base fluid delivery and waste collection
- **Optical modules** - Inbuilt blue light fluorescent detection system provides yield measurements while white light offers real time imaging of experiments in progress for peace of mind

eProtein Discovery™ Cartridge

The eProtein Discovery™ Cartridge is a digital microfluidic-based cartridge powered by electrowetting-on-dielectric (EWOD) technology. Nanoliter sized droplets can be programmed to move across the digital platform by changing electrical pulse sequences generated on the thin film transistor (TFT) and controlled by the software. The cartridge assembly is made out of a heavily pixelated TFT bottom, top glass and encased within a plastic housing. The cell gap, sandwiched between the TFT and top glass layer is filled with oil, an environment where aqueous droplets can be strategically split, moved and merged to create different reaction zones (Figure 2).

Used in combination with the eProtein Discovery Instrument, reagents such as DNA solutions, cell-free expression blends and beads suspensions can be distributed and merged within the cartridge to form expression zones. The instrument drawer provides a platform for retaining beads during purification, enabling binding, washing and elution of target protein. The transparent glass top ensures that fluorescence emission is detected through the optics system located within the instrument. Nanoliter reaction droplets allow for minimal reagent use.

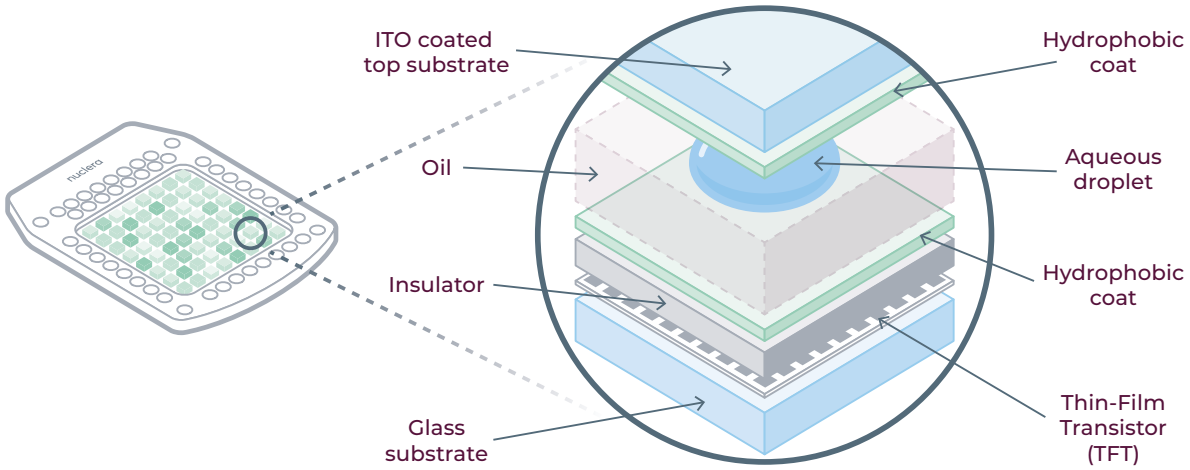
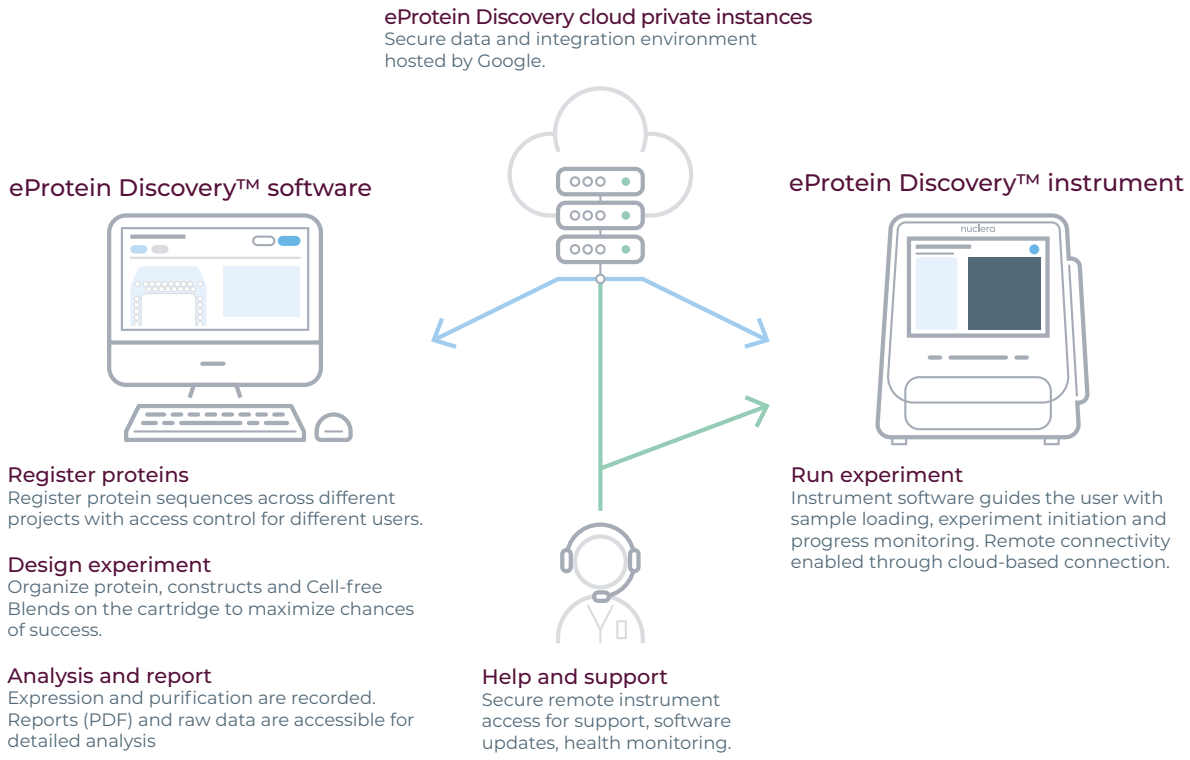


Figure 2. eProtein Discovery Cartridge build depicting the layers of coating and material that make up the digital microfluidic device.

eProtein Discovery™ Software



Intuitive software to guide you
An intuitive cloud-based software provides comprehensive support to users from DNA construct design to experimental run planning. Hosted in a secure cloud-based network, there is no need for complicated downloading or installation. Users can design their experiments from any location with a web browser and network connection.

Secure and intelligent software provides comprehensive support
The software is password protected with user access controls. Users can create projects, design DNA constructs, generate experimental plans, analyze results and generate reports. Intelligent software features include integrated sequence analysis and sequence compatibility checks; hosted on a private server ensuring that uploaded sequence identity remains confidential to the public. Uploaded amino acid sequences are automatically converted to codon optimized DNA sequences with adapter elements added to the N- and C-terminus. Follow through with experimental design by selecting from a list of expression conditions to tailor the run to your specific protein.

Cloud-based software supports seamless transition to instrument
The eProtein Discovery Instrument is also cloud connected. Pre-designed experiments will automatically append to the instrument software accessible through the intuitive touchpad located at the front of the instrument. Users can follow step-by-step instructions on the interface to load and run their experiments. The software on the instrument carries out the user's pre-designed experiment and records the results. A continuous cross-talk between the instrument and cloud-based software enables users to monitor the experiment in real time, at the desktop, from anywhere.

Completed experiment output will be available in the eProtein Discovery Software for data analysis and to generate reports.

Application overview

eGene™ Prep Kit

The eProtein Discovery™ system utilizes linear double stranded DNA (eGene construct) as templates. The eGene constructs can be designed on the eProtein Discovery software and generated via a simple PCR and clean-up process using the eGene Prep Kits. Synthetic gene fragments used as template DNA can be rapidly expanded up to 8 unique constructs with different fusion tags; saving cost and offering convenience. The entire workflow from gene fragment to ready-to-use eGene construct only takes 5 hours (Figure 3b). Users can pick from two eGene Prep Kits depending on experimental needs:

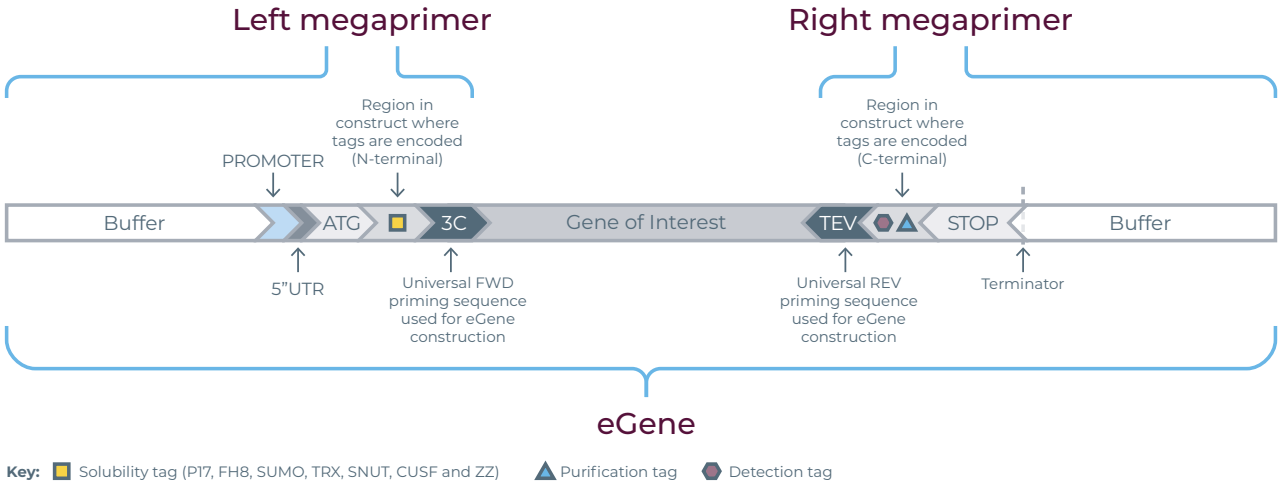
1) **eGene Prep Kit: Solubility Tag Screen**
   Use case: Expand 3, 4 or 6 genes-of-interest into 24 variants with various solubility tags added to the N-terminus and Strep purification and detection tag added to the C-terminus.
   Solubility tag options: P17 tag, FH8 tag, SUMO tag, TRX tag, SNUT tag, CUSF tag, ZZ tag.

2) **eGene Prep Kit: FlexiVariant™ Screen**
   Use case: Maximize gene-of-interest variation (e.g. truncations, domain deletions, mutations, homologs, isoforms) while avoiding solubility tags, or adding your own selection of tags.
   Adds detection and Strep purification tags to the C-terminus.

Re-amplification primers are also provided in each kit for repeat PCR reactions, if necessary.

Purification tag added to the C-terminus ensures that only full length expressed proteins are purified. Detection tag is placed in the C-terminus to ensure that full-length expressed proteins are detected. A 3C protease cleavage site is added to the N-terminus so that the solubility tags can be removed if necessary (Figure 3a).

(a)



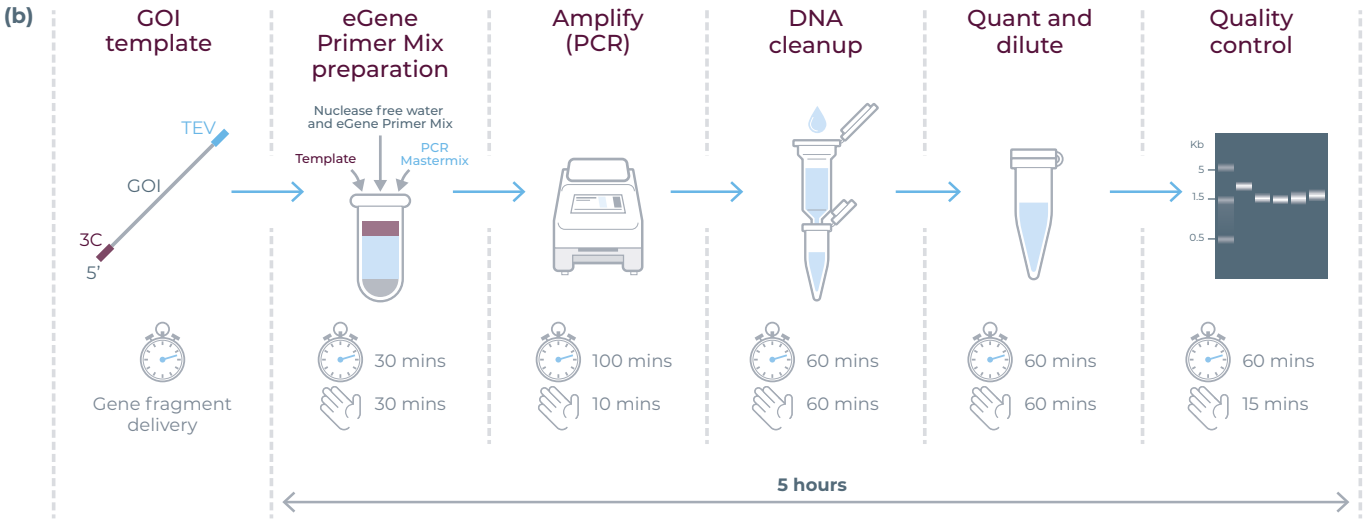


Figure 3. eGene construct configuration and eGene preparation workflow. (a) The gene-of-interest is easily extended with megaprimers coding for the promoter and various fusion tags. Diagram showing positions of each added element (b) eGene workflow summarizing each step and the total reaction time needed to generate eGene constructs using the eGene Prep Kit: Solubility Tag Screen and/or FlexiVariant™ Screen. eGene construct preparation workflow includes (1) DNA construct design on the eProtein Discovery Software and gene fragment ordering, (2) PCR setup, (3) PCR clean-up, (4) DNA quantitation and dilution to 5 nM and (5) agarose gel to verify eGene size and purity.

Cell-Free Protein Synthesis with Nuclera Reagents

Cell-free protein synthesis (CFPS) is an in vitro transcription and translation system that enables efficient protein expression without the need for live cells. Nuclera's Cell-free Core Reagents contain all the elements necessary to express and fold proteins.

Proteins have bespoke expression needs

Correct protein folding depends on one or more interconnecting factors including efficiency of translation mechanisms, the presence of necessary cofactors, chaperones, and a favorable environment that promotes disulfide bond formation. It is estimated that 30% of proteins require cofactors, including metal ions and small organic molecules, for proper folding.[1] Similarly, many proteins require the assistance of transiently associated chaperones to ensure they fold correctly and to prevent aggregation. Yields of recombinant proteins may also be improved through the addition of solubility tags. However, in some instances subsequent removal of these tags is preferred. Cell-free protein synthesis systems offer the flexibility to customize additives present during protein expression, to tailor the conditions needed to properly fold each target protein.

Customizable additives increase chances of success

Nuclera's Cartridge Screen Reagents include the basic Cell-free Core Reagents as well as a range of customizable additives to meet the expression needs of a broad range of proteins. Each cartridge can accommodate up to 8 unique combinations of cell-free blends which include Cell-free Core Reagents and up to 2 additives. If needed, the expression environment can be enhanced with 2x of the same additive* (table 1).

List of additive selection:

Additive options	Benefits
PDI/GSSG	Promote disulfide formation
GSSG	Mimic oxidizing condition in eukaryotic endoplasmic reticulum to promote disulfide bond formation. Also mimics the periplasmic space of prokaryotic cells
TrxB1	Chaperone to promote correct folding and stabilizes correctly folded proteins
DnaK mix	Chaperone that suppress and reverse protein aggregation
Metal ions: $ZnCl_2$, $CaCl_2$, $MnCl_2$,	Metal ions necessary for folding
Cofactor mix (NAD, Acetyl-CoA, FAD, SAM and PLP)	Promote proper folding and stabilizes protein
3C Protease	In situ solubility tag removal
Additive Buffer	Cell-free Core diluting buffer; use when no additive is required

Table 1: Additives selection and their benefits in protein expression. The comprehensive additives list covers most protein expression needs from providing an environment favorable for disulfide bond formation, to adding extra components such as chaperones, co-factors or metal ions to promote proper folding. Solubility tag removal assessment is made possible by the addition of 3C protease. *Using DnaK as a 2x additive may result in a slight decrease in overall expression levels.

1. Bushmarina NA, Blanchet CE, Vernier G, Forge V. Cofactor effects on the protein folding reaction: acceleration of alpha-lactalbumin refolding by metal ions. Protein Sci. 2006 Apr;15(4):659-71. doi: 10.1110/ps.051904206. Epub 2006 Mar 7. PMID: 16522796; PMCID: PMC2242491.

Workflow overview

Expression and purification Screen on cartridge

The eProtein Discovery system will automate the expression screen of 24 different eGene™ constructs against 8 Cell-Free Blends into an expansion of 192 unique expression conditions. Following expression, 30 of the expression droplets will be selected for purification evaluation and yield detection (Figure 4a).

Users can either opt for the system to automatically select the 30 highest-expressing conditions out of the 192 tested conditions, or spread the choice evenly across all tested protein-of-interest (Figure 4a). The system will report on expression yield and purified protein yield using a fluorescent-based complementation assay (Figure 4b). The system can also report on protein yield after 3C protease cleavage, providing insights into protein stability after solubility tag removal. With solubility and purifiability metrics in hand, you can select the recipes to scale up on the bench, next day.

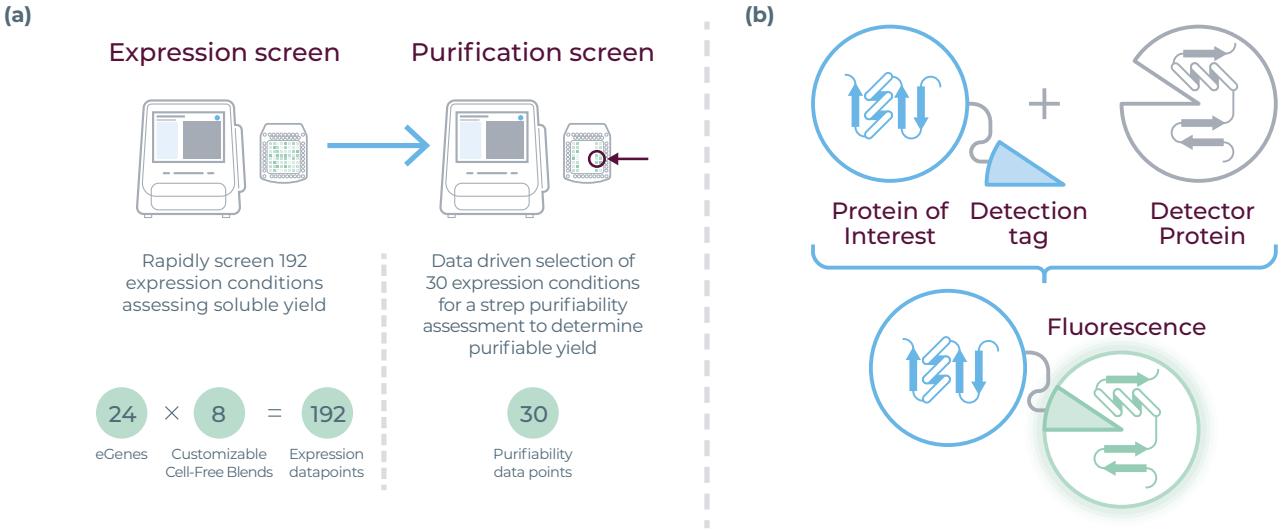


Figure 4: Expression and purification evaluation of target protein. (a) On-cartridge workflow evaluating expression of 192 unique construct and expression condition combinations, to down-selecting 30 for purification assessment. (b) Nuclera proprietary fluorescence complementation technology to detect and report on expression yield. Specificity of fluorescence complementation ensures that only target protein yield is measured.

Scale up expression and purification

The scale up workflow is made easy using the provided Scale-up Kit and Scale-up Additives. The Scale-up Kit contains Cell-Free Core reagents and purification reagents needed for expression and purification in a tube. Scale-up reagents are provided up to 1 mL cell-free protein expression reaction per kit. Guided by the eRecipe™ combinations from the cartridge screen results, one can rapidly produce protein at the bench, at any time.

The scale up workflow is simple and requires no specialized purification columns or equipment (Figure 5).

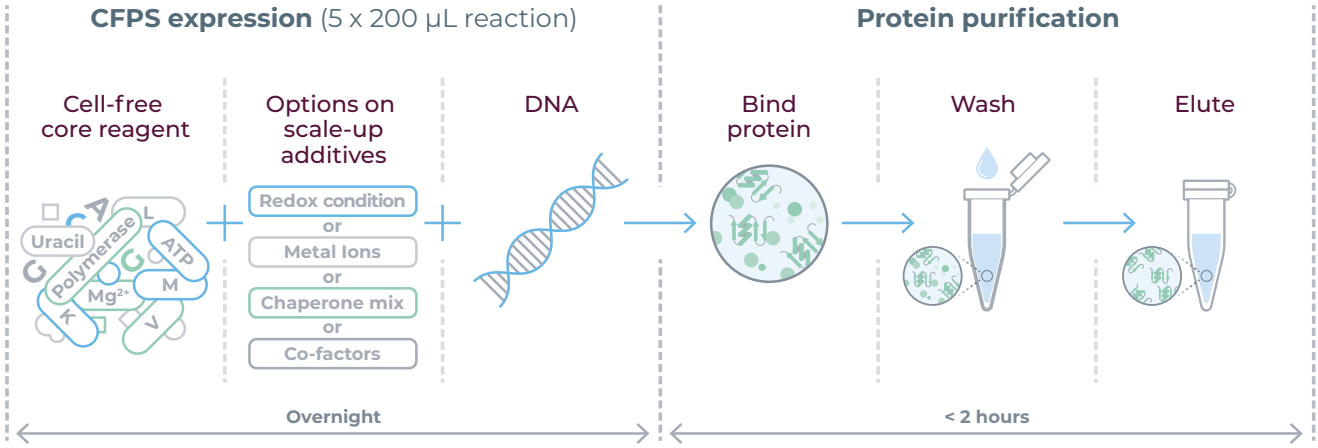


Figure 5. Scale up expression and purification workflow. Using the eRecipe chosen from the cartridge screen results, combine the Cell-free Blends (Cell-free Core + selected Additives) and eGene constructs in an overnight reaction. Purify on beads the next day.

The eProtein Discovery™ system empower scientists to automate 24 constructs expression and purification screen in parallel to guide protein scale up, delivering soluble protein in-hand in less than 48 hours: saving time and minimizing efforts. Nuclera's technology integrates cell-free protein synthesis and digital microfluidics on cartridge, accelerating protein projects through a benchtop, automated, high-throughput protein access system.

System specifications

eProtein Discovery Instrument	Specifications
Dimensions (W x D x H)	375 x 495 x 435 mm (14.7 x 19.5 x 17.1 in)
Bench footprint (W x D x H)	450 x 750 x 450 mm (17.7 x 19.5 x 17.7 in)
Weight	21.6 kg (47.6 lb)
Power supply	100–240 V, ~47–63 Hz
Maximum Power consumption	150 W
Operating temperature	19 – 30 °C

eProtein Discovery Cartridge	Specifications
Multiplex expression	192 unique expression data points in parallel
Bead-based purification	30 selected candidates bound, wash and eluted
High resolution digital droplet control	250,000 individual pixels available on cartridge for programmed droplet movement

Ordering information

Product Code	Instrument bundles
NB5001	eProtein Discovery™ Starter Bundle with Solubility Tag Screen
NB5002	eProtein Discovery™ Starter Bundle with FlexiVariant™ Screen

Product Code	Consumables
NC4001	Cartridge Screen & Scale Bundle with Solubility Tag Screen (2 cartridges)
NC4002	Cartridge Screen & Scale Bundle with FlexiVariant™ Screen (2 cartridges)
NC4003	Cartridge Screen Bundle with Solubility Tag Screen (2 cartridges)
NC4004	Cartridge Screen Bundle with FlexiVariant™ Screen (2 cartridges)
NC4005	Scale-up Bundle (includes Scale-up Kit and Scale-up Additives)
NC3001	eGene™ Prep Kit (Solubility Tag Screen)
NC3002	eGene™ Prep Kit (FlexiVariant™ Screen)
NC3004	Scale-up Kit
NC3005	Scale-up Additives

Product Code	Training and protein evaluation services
NT001	eProtein Discovery™ training
NT002	Technology Access Program (TAP)

Product Code	Service Plans
SPS001	eProtein Discovery™ Silver Service Plan
SPG002	eProtein Discovery™ Gold Service Plan
SPP003	eProtein Discovery™ Platinum Service Plan

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