GenScript CFXpress™ *E. coli* Cell-free Protein Synthesis Kit Instruction Manual

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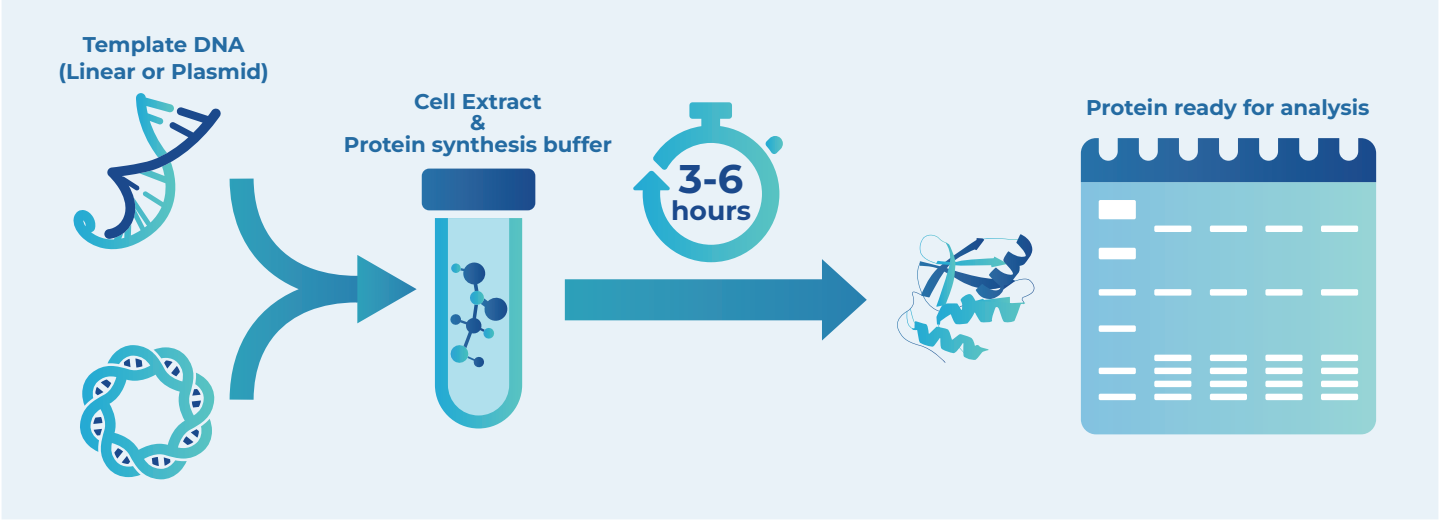
Introduction

The cell-free protein expression is an ***in vitro*** method for protein synthesis that eliminates the need for living cells, reducing the complexity and time typically required for protein production. The cell-free system enables rapid protein expression, simplifies optimization, and is cost-effective for high-throughput applications such as AI-driven protein discovery, protein engineering, and enzyme screening. It is also well-suited for generating proteins used in biophysical and structure-function analyses.

The GenScript CFXpress™ ***E. coli*** Cell-free Protein Synthesis kit is a coupled transcription-translation platform designed to produce proteins from DNA or mRNA templates containing a T7 promoter. The system includes endogenous T7 RNA polymerase and the complete cellular machinery required for protein synthesis - such as ribosomes, translation factors, tRNAs, amino acids, and NTP regeneration components. This system delivers high yield protein expression with the flexibility to scale based on application needs. The synthesized proteins are compatible with downstream process, including purification, SDS-PAGE analysis, western blotting, or direct functional assays.

The kit features a ready-to-use format with a streamlined, user-friendly workflow that minimizes hands-on time. It allows for easy adaptation to various applications, including high-throughput automation workflows, batch reactions, and dialysis-based expression formats. Whether for rapid prototyping in small volumes or scaled-up synthesis for functional analysis, the system supports flexible and efficient integration into your research pipeline.

General workflow chart



Kit Component

Reaction Size	Component	Quantity	Storage	Shipping	Catalog #
1 mL (20 reactions)	Cell Extract 185 µL (red cap)	2	-80°C	Dry ice	RP-00005-1
	Protein Synthesis Buffer 300 µL (translucent cap)	2	-20°C or -80°C		
	Control Plasmid (pCFe-sfgfp) 7.5 µg, 0.5 µg/µL (blue cap)	1	-20°C or -80°C		

Reaction Size	Component	Quantity	Storage	Shipping	Catalog #
5 mL	Cell Extract 370 µL (red cap)	5	-80°C	Dry ice	RP-00005-5
	Protein Synthesis Buffer 600 µL (translucent cap)	5	-20°C or -80°C		
	Control Plasmid (pCFe-sfgfp) 15 µg, 0.5 µg/µL (blue cap)	1	-20°C or -80°C		

Required Material and Equipment

1. Nuclease-free reaction vessels - sterile microcentrifuge tubes, PCR strip tubes, or lidded multi-well plates,depending on your desired reaction volume.
2. Temperature-controlled incubator - A thermal cycler with a heated lid, a shaking incubator, or any enclosed system capable of maintaining a consistent temperature is suitable.
3. Nuclease-free water - Essential for dilutions and maintaining reaction integrity.
4. DNA template - Provide either linear or plasmid DNA that encodes your protein of interest.

Optional Reagent:

- Chaperones or Folding Additives (for improving protein folding)

Template Guidelines

Template Types

PCR products, linear or circular plasmid DNA, or mRNA can be used as a template. Higher yields are often obtained with circular plasmid DNA than using linear templates. When using linear templates, a DNA exonuclease inhibitor, such as GamS Nuclease Inhibitor, can be added to stabilize linear DNA and achieve close to plasmid-level yields.

Template Purity & Concentration

Template purity is important for successful in vitro transcription/translation; use commercial miniprep kit to isolate plasmid or linear DNA. DNA templates should have a OD260:280 ratio of 1.8-2.2, and OD260:230 ratio of 2.0-2.3. The optimal plasmid DNA template can range from 10-20 ng/μL.

Template Design

For convenience, the Genscript pCFe-sfgfp Control Plasmid can be used as a cloning vector (Figure 1). This high copy vector contains the required T7 promoter, ribosome binding site, T7 terminator elements. The superfold-GFP gene can be replaced with other genes of interest.

Figure 1. Schematic representation of the control Vector construct

If you are designing linear DNA template, the template must contain required T7 promoter, ribosome binding site, T7 terminator elements. The PCR primers used for linear DNA template amplification can be designed either based on sequence of circular plasmid or designed DNA template.

Figure 2. Schematic DNA template design for cell-free expression

Protocol

Protein Synthesis Procedure

Typical reactions are conducted in a 50 µL volume, but this can be adjusted based on experimental needs: for volumes between 20 and 500 µL, use sterile, nuclease-free 1.5- or 2-mL microcentrifuge tubes; for reactions larger than 500 µL, use lidded multi-well plates compatible with your incubator or shaker.

To prepare the reaction, follow the steps below:

1. Thaw all components on ice.
2. Gently vortex Cell-Extract and Protein Synthesis Buffer to ensure they are well mixed.
3. On ice, combine the reagents in a suitable nuclease-free vessel according to the following guidelines:

Component	50 µL reaction	M µL reaction
Cell Extract (red cap)	16.5 µL	0.333 x M µL
Protein Synthesis Buffer (transluscent cap)	26 µL	0.517 x M µL
Plasmid Template (or Control Plasmid)	10-20 ng/µL (final concentration)	10-20 ng/µL (final concentration)
Nuclease-free Water	Adjust to final volume	Adjust to final volume
Total Volume	50 µL	M µL

4. Incubate the reactions at 30°C with vigorous shaking for 3 to 6 hours, or alternatively, incubate at 25°C overnight.
5. Stop reaction by placing the reaction system on ice.
6. Analyze the reaction using your preferred method or store samples at –20°C for future use.

Practical Tips

The cell extract must be stored at -80°C. All other kit components can be stored at –20°C or -80°C.

Components may lose activity if stored improperly or subjected to repeated freeze-thaw cycles. To prevent this, aliquot reagents according to your reaction volume requirements.

Keep all reagents on ice during thawing and reaction assembly, and minimize freeze-thaw cycles to maintain reagent integrity.

4. Plasmid may contain inhibitors of transcription or translation; use commercial miniprep kit to purify plasmid or linear DNA templates.
5. To avoid nuclease contamination, always wear gloves and use nuclease-free water, pipette tips, and microcentrifuge tubes.
6. Verify that the coding sequence is correct and in-frame to ensure proper protein translation.
7. Protein bands smaller than the expected full-length product may be proteolytic fragments formed during the reaction. Including protease inhibitors can help mitigate this issue.
8. DTT is omitted from the cell extract and protein synthesis buffer. For expression of proteins without disulfide bonds, adding 2 mM DTT to the reaction mixture is recommended to enhance protein yield.
9. As reaction volume increases, the reduced headspace may require enhanced oxygen exchange. This can be addressed by puncturing the cap or using a breathable film.

Tips for Protein Expression Optimization

1. The proteins originated from other species might require codon optimization for expression in the *E. coli* system.
2. Incubating at lower temperatures (down to 25°C) for longer periods of time (up to 16 hours), may increase the soluble protein level.
3. 10-20 ng/μL of plasmid in reaction is recommended. Optimization with different amounts of template DNA may improve the yield of a particular target protein.
4. For proteins with disulfide bonds, supplement the reaction mixture with DsbC and modify the redox level.
5. The protein yield is dependent on the nature of the target protein. The system routinely produces between 0.05 to 1 mg/mL. The conditions for a given target will require optimization of the incubation temperature and time, as well as the concentration of the template and supplements.
6. Reactions can be scaled up or down linearly with suitable vessels; it is necessary to keep good aeration using a plate shaker or thermal mixer. When working with small reaction volumes do not allow evaporation to occur.

Appendix

How to evaluate/analyze positive control

The Cell-Free Expression Kits include the pCFe-sfGFP Control Plasmid, which encodes super-fold green fluorescent protein (sf-GFP). The control plasmid can be used to evaluate the performance of the cell-free protein expression. The GFP protein can served as a qualitative or quantitative indicator of successful protein expression. GFP fluorescence can be recorded using microplate reader with excitation wavelength of 488 nm and emission wavelength of 525 nm.

Method for preparing reaction for SDS-PAGE

Protein produced by the GenScript CFXpress *E. coli* Cell-free Protein Synthesis System can be directly loaded onto an SDS-PAGE gel without the need for acetone or TCA precipitation.

1. Combine 5 µL of reaction mixture with 5 µL of SDS-PAGE loading buffer, and 10 µL H2O. Also prepare a negative control sample.
2. Incubate at 100°C for 3-5 minutes.
3. After a quick microcentrifuge spin, load 4-6 µL sample directly on to the gel.
4. Run the gel according to the manufacturer's recommendations.
5. Stain with Coomassie Blue or another stain as directed or proceed to western blot.

After staining, the target protein is typically observed as a unique band, absent in the negative control reaction. Sometimes, the target protein might comigrate with endogenous protein in cell lysate, therefore enhancing the comigrating band.