

Cell-Free Protein Synthesis Kit Pro (Lyophilized)

REF: EG25303-S/M

Storage Condition

4°C transportation.

Store at -20°C . Valid for 12 months.

After reconstitution, store at -20 °C for no more than 1 month, and at -80°C for no more than 12 months.

Components

Component	EG25303S	EG25303M
Cell-free system solution A Pro (Lyophilized)	1 vial (300 µl after reconstitution)	1 vial (1.5 ml after reconstitution)
Cell-free system solution B (Lyophilized)	1 vial (600 µl after reconstitution)	1 vial (3 ml after reconstitution)
CFPS-Control Plasmid	2 µg	2 µg

Description

The Cell-Free Protein Synthesis Kit is a product for in vitro protein synthesis based on E. coli lysate. Coupled transcription/translation systems contain an RNA polymerase and the necessary cellular machinery needed to direct protein synthesis (e.g., ribosomes, translation factors and tRNAs). Supplements such as amino acids, an energy source and NTPs complete the system. Proteins are expressed using DNA or RNA as templates. Cell-free protein expression, independent of viable cells, enables rapid, flexible and high-yield protein expression, with numerous advantages over traditional recombinant expression systems:

1. Time saving. Express target proteins in 1~2 hours, with peak yield achieved in 8~24 hours.
2. High protein expression level, up to more than 3 mg/ml.
3. Easy operation. Add DNA or RNA templates to the reaction system, and the reaction can be performed using 96-well plates or centrifuge tubes.

Note: The product contains chaperone proteins, which is suitable for expressing insoluble protein.

Application

The product is restricted for scientific research and shall not be used for clinical diagnosis or treatment, nor for food or pharmaceuticals.

Protocol

1. Gene construction

For convenience, the pJL1-sfGFP included in this kit can be used as a cloning vector (Obtain the plasmid profiles by scanning the QR code on the outer packaging). This high copy vector contains the required T7 promoter, ribosome binding site, T7 terminator elements. It is also compatible with pET series plasmids such as pET-9a and pET-23a, which contain the T7 promoter but no lactose operon (lac).

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TAATACGACTCACTATA GGGAGACCACAACGGTTTCCC TCTAG
AAATAATTTTGTTTAACTTTAAG AAGGAGAATATACC ATG.....
.....TAAGTCGACCGGCTGCTAACAAAGCCCGAAA
GGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAA CTAGC
ATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTGG
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Note:
— T7 promoter
— g10 stem-loop
— ribosome binding site RBS
— Target gene
— T7 terminator

Figure 1 Required Elements for Template DNA.

Note: Plasmids containing the lac operon (such as pET28a) will significantly affect the yield and are not recommended for direct use.

The schematic diagram of the DNA sequence of the positive plasmid is as follows:

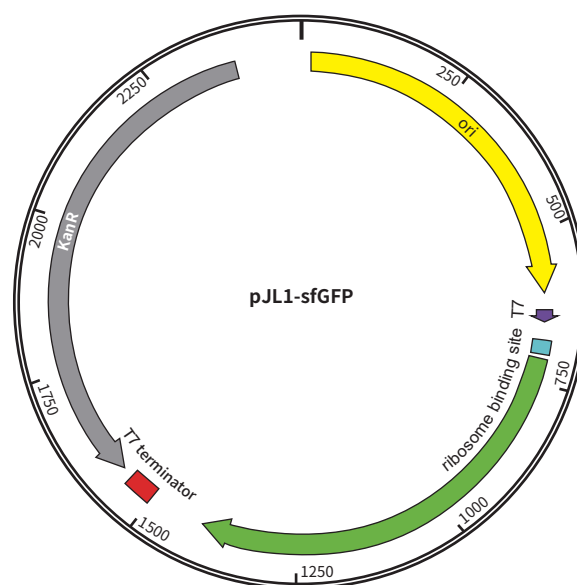


Figure 2 Schematic of the Control Plasmid.

2. Template preparation

DNA or mRNA can be used as templates. The DNA templates can be PCR products, linear or circular plasmid DNA, mRNA, or products amplified via RCA (Rolling Circle Amplification) using phi29.

- (1) Plasmids: Plasmids are either directly synthesized by gene synthesis companies or obtained via subcloning and extracted using column purification.
- (2) PCR products: The forward primer is designed approximately 200 bp upstream of the T7 promoter, and the reverse primer approximately 200 bp downstream of the T7 terminator. After PCR amplification of the template, the linear DNA fragments can be directly used in the cell-free reaction system without purification. The 200 bp bases upstream and downstream serve to protect the linear DNA fragments from degradation by endogenous exonucleases.
- (3) RCA products: Rolling circle amplification (RCA) is performed using phi29 polymerase and random hexamers. DNA products can be directly used in the cell-free reaction system.
- (4) PCR and RCA can be combined with Golden Gate and Gibson Assembly, which will greatly enhance the speed and throughput of DNA template preparation.
- (5) DNA templates must be accurately quantified before use. It is recommended to use high-quality plasmid extraction kits for plasmid isolation to avoid introducing RNase A. For plasmids provided by gene synthesis companies, it is emphasized that column purification must be performed, otherwise, they cannot be directly used in cell-free reactions.

3. Standard Protocol

- (1) Reconstitute the lyophilized solution A with 300 μ l and lyophilized solution B with 500 μ l of Nuclease-Free water respectively (or reconstitute the lyophilized solution A with 1.5 ml and solution B with 2 ml of RNase Free Water respectively) and then mixing completely.
- (2) Calculate the required volumes of Solution A and Solution B (at a volume ratio of 1:2) in nuclease-free 2 ml microcentrifuge tubes. Reactions are typically 50 μ l but can be scaled down or up, as needed. Components can be pre-assembled to create a master mix for a desired number of reactions. To prevent nuclease contamination, wear gloves and use nuclease-free tubes and tips. Keep all reagents on ice before and during the assembly of reactions and avoid repeated freeze-thaw cycles of the tubes. Combine reagents in a 2 ml microcentrifuge tube on ice as follows:

Table 2 Reaction System

Reagent	Final Concentration	50 μ l reaction system	100 μ l reaction system
Cell-free system solution A Pro	30%	15 μ l	30 μ l
Cell-free system solution B	60%	30 μ l	60 μ l
Template	5~10 μ g/ml	5~10 μ g/ml	5~10 μ g/ml
Nuclease-Free water	\	Up to 50 μ l	Up to 100 μ l

- (3) Add DNA template to the reaction system. The recommended final concentration is 5~10 μ g/ml, and the amount can be optimized.
- (4) Place the reaction vessel in a shaker or thermostatic mixer for cell-free protein expression, with the temperature being 25~30°C. Lowering the incubation temperature to 25°C can help the synthesis of some target proteins. Maximum protein yield is generally achieved after approximately 8 hours of reaction, and an overnight reaction of 16 hours is also feasible. If reduce reaction temperature, the reaction time should be appropriately extended.
- (5) Typical reaction conditions use a 100 μ l reaction volume in 2 ml microcentrifuge tube. Reactions can be scaled up or down linearly. When working with large reaction volumes it is necessary to include sufficient headspace and/or aeration, such as shake flasks, with the shaker speed maintained at 200 rpm.

4. Detection

After the reaction, take 1 μ l of the reaction solution (for detecting total proteins) or reaction supernatant (for detecting soluble proteins) to perform SDS-PAGE electrophoresis for detecting the expression of the target protein.

5. Positive Control

This kit contains the sf-GFP plasmid (a green fluorescent protein) as a positive control, allowing direct visual observation of reaction results. Upon successful expression of sf-GFP, the system will exhibit obvious green fluorescence. For accurate quantification of sf-GFP, a microplate reader can be used for detection (Ex/Em = 485/528 nm).