# Cell-Free Protein Synthesis Kit Pro

REF: EG25301-S/M

## Storage Condition

Store at -80℃ . Valid for 12 months.

Dry ice transportation. After opening, these components should be stored at -80℃ .

Avoid repeated freezing and thawing. According to the reaction volume, solution A and solution B can be aliquoted separately and rapidly frozen in liquid nitrogen, then stored at -80℃ .

## Components

| Component | EG25301S | EG25301M |
|---|---|---|
| Cell-free system solution A Pro | 300 μl | 1.5 ml |
| Cell-free system solution B | 600 μl | 3 ml |
| 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).

TAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAG
AAATAATTTTGTTTAACTTTAAGAAGGAGAATATACC**ATG**.....
.....**TAA**GTCGACCGGCTGCTAACAAAGCCCGAAA
GGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGC
ATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

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:

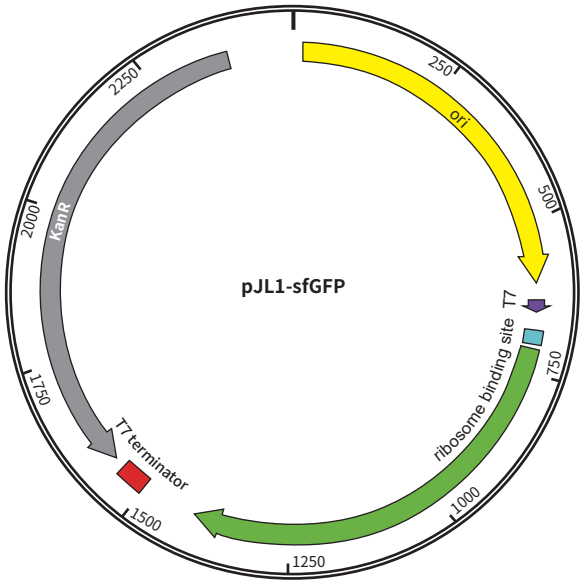


Figure 2 Schematic of the Control Plasmid.



Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com

## 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) 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 µl 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 µl reaction system | 100 µl reaction system |
|---|---|---|---|
| Cell-free system solution A Pro | 30% | 15 µl | 30 µl |
| Cell-free system solution B | 60% | 30 µl | 60 µl |
| Template | 5~10 µg/ml | 5~10 µg/ml | 5~10 µg/ml |
| Nuclease-Free water | \ | Up to 50 µl | Up to 100 µl |

(2) Add DNA template to the reaction system. The recommended final concentration is 5~10 µg/ml, and the amount can be optimized.

(3) Place the reaction vessel in a shaker or thermostatic mixer for cell-free protein expression, with the temperature being 25~30℃ . Lowering the incubation temperature to 25℃ 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.

(4) Typical reaction conditions use a 100 µl 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 µl 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).



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