Cell-Free Protein Synthesis Kit Pro (Lyophilized)

REF: EG25303-S/M

Storage Condition

4℃ transportation.

Store at -20℃ . Valid for 12 months.

After reconstitution, store at -20 ℃ for no more than 1 month, and at -80℃ 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 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 can be expressed using DNA or RNA as templates. Cell-free protein synthesis does not rely on living cells, enabling rapid and flexible 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 expression level. Up to more than 3 mg/ml.

3. Easy operation. Only DNA or RNA template needs to be added, and the reaction can be performed using 96-well plates or centrifuge tubes.

Note: This product contains the chaperone proteins GroEL (~60 kDa) and GroES (~10 kDa), representing the most extensively characterized chaperone system currently under research. They create a closed folding chamber that provides an optimal environment for unfolded proteins, reducing misfolding and aggregation, thereby increasing the probability of soluble expression. However, protein solubility is influenced by multiple factors (e.g., inherent protein structure, expression system, reaction conditions, etc.). While this product can significantly improve folding efficiency, soluble expression is not guaranteed for all proteins.

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 decrease the yield and are not recommended as template.

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

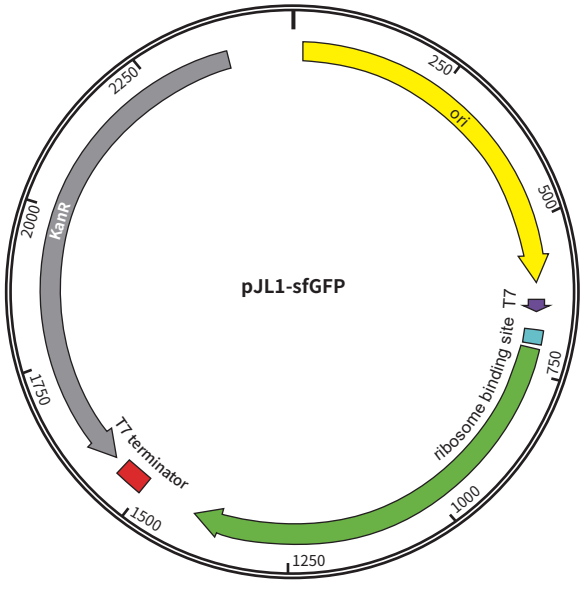

Figure 2 Schematic of the Control Plasmid.

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2. Template preparation

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

(1) Plasmids: Directly synthesized, or obtained via subcloning and purified 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, the linear DNA fragments can be directly used as template 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.

Note: Accurately quantify the DNA template before use.Use a high-purity plasmid extraction kit containing separate deproteinization Wash Buffer, avoiding introduction of RNase A. Emphasize that plasmids provided by the company must be purified using column chromatography, otherwise, they cannot be directly used for cell-free reactions. For column-purified DNA templates, elution with nuclease-free water is recommended.

3. Standard Protocol

(1) Reconstitute the lyophilized solution A with 300 μl and lyophilized solution B with 500 μl 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) Reactions are typically 50 μl but can be scaled down or up, as needed. 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 1.5 or 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

(3) The recommended final concentration of DNA template is 5~10 μg/ml.

(4) Incubate reactions at 25~30 ℃ , with vigorous shaking. Lowering the incubation temperature to 25 ℃ can help the synthesis of some 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 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 total solution (for total proteins) or supernatant (for 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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