Cell-Free Protein Synthesis Kit (Kcr)

REF: EG25332S

Storage Condition

Store at -80℃ . Valid for 12 months.

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

To avoid multiple freeze-thaw cycles, Solution A and Solution B can be aliquoted according to reaction volume, snap-frozen in liquid nitrogen, and then stored at -80℃ .

Components

Component	Amount
Cell-free system solution A	300 μl
Cell-free system solution B	400 μl
CFPS-Control Plasmid	2 μg
Nε-crotonyl-L-lysine Kcr (25×)	60 μl
tRNA (25×)	60 μl
Aminoacyl-tRNA synthetase (50×)	40 μl

Description

Cell-Free Protein Synthesis Kit is an extract-based transcription/translation system derived from E. coli cells. It enables the site-specific incorporation of the Nε-crotonyl-L-lysine (Kcr) into the target protein by utilizing the amber stop codon TAG in the DNA sequence. The kit contains three additional components: the noncanonical amino acid Kcr, tRNA, and aminoacyl-tRNA synthetase. Dosage can be optimized according to requirements.

Note: This product under this catalog number enables the expression of disulfide bond-rich 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:

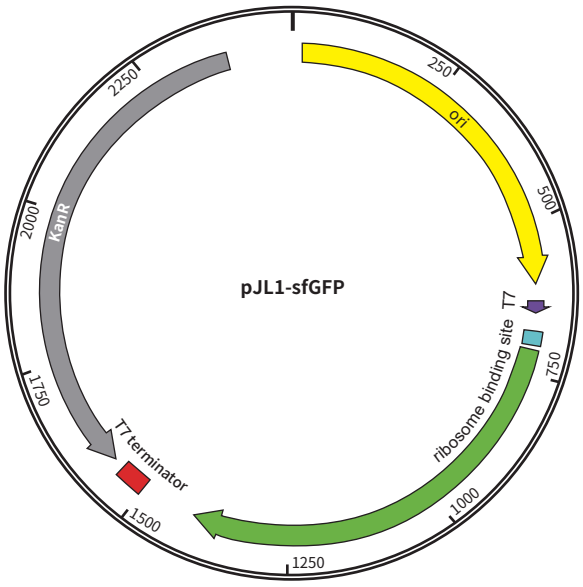

Figure 2 Schematic of the Control Plasmid.

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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) 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	30%	15 μl	30 μl
Cell-free system solution B	40%	30 μl	40 μl
Template	5~10 μg/ml	5~10 μg/ml	5~10 μg/ml
Nε-crotonyl-L-lysine Kcr (25×)	1×	2 μl	4 μl
tRNA (25×)	1×	2 μl	4 μl
Aminoacyl-tRNA synthetase (50×)	1×	1 μl	2 μl
Nuclease-Free water	\	Up to 50 μl	Up to 100 μl

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

(3) 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.

(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 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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