

Tube Protein Synthesis Kit

Catalog No.: 2902-020 Size: 20 rxns

Catalog No.: 2902-100 Size: 100 rxns

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	2902-020	2902-100
Tube Protein Synthesis solution A (Lyophilized)	1 vial (300 µl after reconstitution)	1 vial (1.5 ml after reconstitution)
Tube Protein Synthesis solution B (Lyophilized)	1 vial (600 µl after reconstitution)	1 vial (3 ml after reconstitution)
Control Plasmid	2 µg	2 µg

Description

Tube 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. Tube Protein Synthesis Kit 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 is not suitable for expression of disulfide-rich proteins. If needed, please contact us for other REF NO..

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
AAATAATTTTGTTAAC TTTAAG AAGGAGAATATACC ATG.....
.....TAA GTCGACCGGCTGCTAACAAAGCCCGAAA
GGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAA CTAGC
ATAACCCCTTGGGGCCTCTAACCGGGTCTTGAGGGGTTTTTTG
  
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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 decrease the yield and are not recommended as template.

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

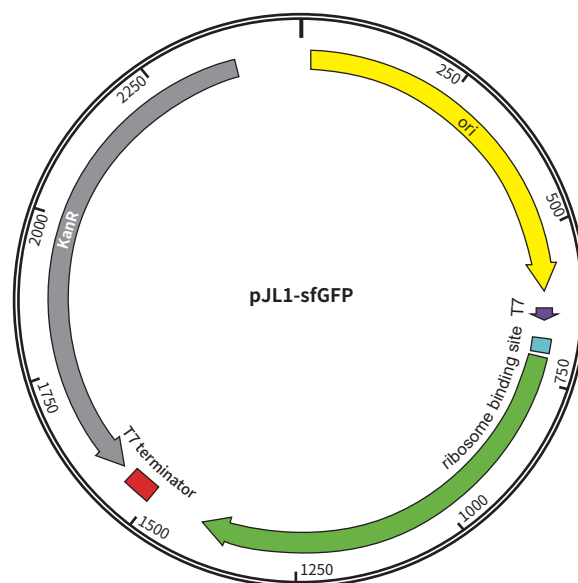


Figure 2 Schematic of the Control Plasmid.

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.
- (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. Plasmids provided by gene synthesis companies must be purified using a column method; otherwise they cannot be directly used as template.

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) Reactions are typically 50 μ l 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 μ l reaction system	100 μ l reaction system
Tube Protein Synthesis solution A	30%	15 μ l	30 μ l
Tube Protein Synthesis 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) The recommended final concentration of DNA template is 5~10 μ g/ml.
- (4) Incubate reactions at 25~30 $^{\circ}$ C, with vigorous shaking. Lowering the incubation temperature to 25 $^{\circ}$ C 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 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 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).