Cell-Free Protein Expression Kit (248507)

Introduction to Cell-Free Expression Systems

Concept

A cell-free protein expression system uses exogenous mRNA or DNA as templates and synthesizes proteins in vitro using cell extracts or reconstituted systems that contain the enzymes, cofactors, amino acids, substrates, and energy required for protein expression. Because the reaction is not limited by cellular metabolism or the closed intracellular environment, proteins can be produced rapidly and in high yield.

Common cell-free protein expression systems include the Escherichia coli system, insect cell lysate system, wheat germ system, and rabbit reticulocyte system.

Advantages

- Small reaction volume allows parallel synthesis of multiple different proteins.
- Non-natural amino acids or isotope-labeled amino acids can be added to express specialized proteins.
- No cell structure limitation, making it suitable for proteins that are toxic to host cells.
- Short reaction cycle supports high-throughput ligand screening and proteomics research.
- No plasmid transformation, cell culture, collection, disruption, or centrifugation is required.
- Improved expression for proteins that are difficult to produce in ordinary cell lines, including highly hydrophobic and multi-transmembrane proteins.
- The open reaction system allows easy adjustment of reaction conditions for transcription, translation, and post-translational regulation, while helping avoid inclusion body formation.

Escherichia coli Cell-Free Expression System

The Escherichia coli cell-free protein expression system is widely used because it has a clear genetic background, high target gene expression, a short preparation cycle, low cost, strong contamination resistance, and good scalability. It is suitable for proteins from many species and is widely applied in protein interaction studies, high-throughput screening, and gene circuit research.

This system uses coupled transcription and translation to produce active recombinant proteins in vitro. It removes labor-intensive steps from traditional intracellular protein production, including transformation, cell culture, and expression optimization. Within a few hours, it can generate high-yield, high-quality proteins from circular plasmid DNA or linear DNA templates for screening, structural analysis, or functional analysis.

Kit Overview

This kit enables rapid and efficient expression of target proteins from plasmid or linear DNA templates using a one-pot coupled transcription-translation reaction. Except for the target protein gene, the system contains the enzymes, energy sources, amino acids, nucleotides, inorganic salts, and other components required for in vitro transcription and translation.

Main Components of the Expression System

- Optimized Escherichia coli extract to improve DNA stability during transcription and translation and increase soluble protein yield.
- Optimized reaction buffer with an ATP regeneration system for continuous energy supply.
- Amino acids at optimized concentration ratios to provide sufficient substrate for protein synthesis.
- An optimized expression vector containing the GFP gene as a positive control for visual confirmation of protein expression.

Product Features

- Fast: target protein synthesis can be completed in 2-16 hours.
- Flexible templates: supports linear templates, plasmid templates, and PCR products.
- High yield: protein preparation yield up to 3 mg/mL.
- Protein co-expression: multiple target proteins can be expressed in the same reaction.
- Simple operation: prepare proteins by mixing the reaction components with the template.
- High-throughput: suitable for 96-well plate reactions and scalable after positive expression is confirmed.
- Broad applicability: suitable for many protein types, including disulfide-bond-rich proteins and membrane proteins.

Before You Start

> If you use the plasmid DNA template provided with this kit to construct a target gene expression vector, the template must contain a T7 promoter, a start codon, and a prokaryotic ribosome binding site (RBS) upstream of the target gene.

Recommended Template Design

- The target gene should be located downstream of the T7 promoter and RBS and must contain an ATG start codon and a stop codon.
- The sequence between the RBS and the ATG start codon should be 7-9 nt for optimal translation efficiency. This sequence does not need to be specific.
- Upstream of the T7 promoter, include at least 6-10 nt of sequence to support efficient promoter binding. This is required for linear PCR products and does not need to be specific.
- After the T7 promoter, include at least 15-20 nt to allow formation of a potential stem-loop structure.
- A T7 terminator located 4-100 nt downstream of the target gene can effectively terminate transcription and help maintain transcript stability.

Package Contents

Package size: 1 mL

Part No.	Component	Specification
BR2108606.1	Component A	20 rxns
BR2108606.2	Component B	20 rxns
BR2108606.3	Positive control plasmid (PC)	4 μg
BR2108606.m	Manual	1 copy

Quality Standards and Safety

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Component A	---	---
Component B	---	---
Positive control plasmid (PC)	---	---

Transportation and Storage

Transportation: Ship on dry ice.

Storage: After opening, store the cell-free protein expression reaction components at -80°C for long-term frozen storage. Fresh preparation for immediate use is recommended.

If reagents must be aliquoted after thawing, snap-freeze the aliquots in liquid nitrogen and then store them at -80°C. Avoid repeated freeze-thaw cycles.

Control Plasmid Map

Sequence upstream of the start codon (5'→3'): ACTTT AAGAA GGAGA TATAC AT ATG

Sequence downstream of the stop codon (5'→3'): TGA GT CGACC GGCTG CTAAC AAAGC

Instructions for Use

1. Template Preparation

This kit can use circular or linear DNA as the expression template.

Circular plasmid template: Use a plasmid containing T7 promoter elements as the vector backbone to construct the expression vector. Plasmid DNA can then be used as the cell-free protein expression template. pID and pJL1 plasmids are recommended as backbone vectors. pET plasmids can also be used, but the presence of the lac operon may reduce protein expression.

Linear DNA template: Use overlap PCR to prepare a linear DNA expression template with T7 transcription elements flanking the target gene.

> The cell-free protein expression system is highly sensitive to nucleases. Some plasmid extraction reagent kits may leave residual nucleases. Consider using PCR purification kits to remove residual nucleases.

2. Preparation Before the Cell-Free Reaction

1. Calculate the required amounts of Component A and Component B.
2. Place the reagents to be used on ice.
3. Perform the experiment on ice to help prevent reagent inactivation.
4. Avoid repeated freeze-thaw cycles of Components A and B.
5. If needed, mix 1 tube of Component A with 1 tube of Component B thoroughly, aliquot as required, snap-freeze in liquid nitrogen, and store at -80°C. If aliquoted at 37 µL per tube, each aliquot may be used in a 50 µL reaction system.
6. Wear gloves and masks, and use nuclease-free reagents, containers, and pipette tips throughout the procedure.

3. Setting Up the Cell-Free Reaction

1. Add each reagent to the reaction vessel according to the reference reaction system and mix by pipetting up and down. Avoid vigorous shaking.
2. Place the reaction vessel on a standard shaker at 180 rpm and 25°C, or on a benchtop constant-temperature mixer at 600 rpm and 25°C.
3. Incubate for 2-16 hours. Most proteins can be fully expressed within 6-8 hours, but some proteins may benefit from a 16-hour reaction.
4. For the first experiment, an overnight reaction of 16 hours is recommended to ensure complete expression of the target protein.

4. Reference Reaction System

The reaction system can be scaled up or down proportionally as needed.

Component	Amount
Component A	20.5 µL
Component B	16.7 µL
DNA template	Final concentration 20 ng/µL
ddH2O	Make up to 50 µL
Total volume	50 µL

5. Recommended Reaction Vessels

Reaction Vessel	Volume
96-well plate	50 µL
48-well plate	100 µL
24-well plate	200-300 µL
6-well plate	500-1000 µL

Reaction Vessel	Volume
250 mL conical flask	10-100 mL

Precautions

1. This kit is based on a prokaryotic expression system. It can express prokaryotic proteins, including bacterial proteins, viral proteins, proteins containing disulfide bonds, and eukaryotic proteins that do not require post-translational modification.
2. The T7 promoter is preferred as the transcription initiation element.
3. Mix thoroughly by pipetting before use and try to avoid bubble formation.
4. Factors affecting protein yield include protein size, DNA template quality, the target gene sequence, and the position of the target gene on the DNA template relative to the T7 promoter and RBS binding site.
5. Even with optimally prepared and purified DNA templates, protein size and sequence can still affect yield. Expression conditions may require experimental optimization.

Quick Guide to Cell-Free Reactions

Preparation of the Reaction Mixture

1. Place the required reagents on ice and allow them to thaw completely.
2. Components A and B can be mixed thoroughly and aliquoted as needed. Snap-freeze any remaining reagents in liquid nitrogen and store them at -80°C to avoid repeated freeze-thaw cycles.
3. Add each reagent according to the recommended reaction system and mix thoroughly by pipetting up and down. Avoid vigorous shaking. The recommended final template concentration is 10-40 ng/μL.

Starting and Running the Reaction

1. Place the prepared reaction mixture in the reaction device and incubate for 2-16 hours. The reaction time may be shortened or extended as needed. For the first reaction, an overnight incubation of 16 hours is recommended to ensure complete expression of the target protein.
2. Recommended equipment settings: thermostatic mixer at 25°C and 600 rpm, or shaker at 25°C and 180 rpm.
3. Centrifuge at 4°C, 10000g, for 2 minutes.
4. Collect the supernatant for analysis.

Analysis of Protein Expression Results

GFP is used as the positive control. It can be detected with instruments capable of reading fluorescence signals, such as a microplate reader or fluorescence microscope, or by direct visual observation of fluorescence in the reaction solution.

GFP detection: ex/em 485/510 nm

Target protein expression may be analyzed by polyacrylamide gel electrophoresis, Western immunoblot analysis, or other suitable methods. For polyacrylamide gel electrophoresis, it is recommended to use 1 μL of supernatant.