Cell-Free Protein Synthesis Kit Max

Cat. No.: C1522808 | Pack size: 20 reactions; 100 reactions | Storage: -80°C; avoid freeze/thaw

Product Information

Attribute	Value
Pack Size(s)	20 reactions; 100 reactions
Specifications & Purity	BioReagent, for protein analysis
Grade	BioReagent, for protein analysis
Application	Protein Expression
Storage Conditions	Store at -80°C, Avoid repeated freezing and thawing
Shipped In	Dry ice packs + Cold packs
Stability And Storage	Each component has a shelf life of 1 year under corresponding storage conditions.

Product Description

The kit is a product for in vitro protein synthesis based on Escherichia coli cell lysate. This kit utilizes active components in the cell lysate, including ribosomes, translation factors, and enzymes, supplemented with energy sources, nucleotides, amino acids, inorganic salts, etc., to reconstitute a transcription-translation system in vitro. It expresses target proteins using DNA or RNA as templates.

Cell-free protein expression enables rapid, flexible and high-yield protein production independent of living cells, and offers numerous advantages over traditional recombinant expression systems:

1. Fast protein expression: Target protein can be produced within 1-2 hours, and maximum yield is achieved in 8-24 hours.
2. High protein yield: Up to more than 3000 μg/mL protein.
3. Simple and flexible reaction: Only DNA or RNA template needs to be added to the reaction system; reactions can be performed in 96-well plates or centrifuge tubes.

Note: This product can express conventional proteins and is particularly suitable for expressing proteins containing disulfide bonds. After opening, store all cell-free protein expression components

at -80 °C. To avoid repeated freeze-thaw cycles, aliquot Solution A and Solution B separately according to usage amount, snap-freeze in liquid nitrogen, and then store at -80 °C.

Application

This product is limited to scientific research use by professional personnel. It shall not be used for clinical diagnosis or treatment, nor for food or pharmaceutical applications.

Protocol

1. Gene Construction

Gene construct design is critical for efficient protein expression. The construction strategy shown below is recommended. The target gene can be cloned into the positive control plasmid supplied with this kit (plasmid map is available via the QR code on the outer package label). The kit is also compatible with pET-9a, pET-23a, and other pET-series plasmids containing the T7 promoter but without the lac operator.

Schematic diagram of the positive control plasmid DNA sequence is shown below:

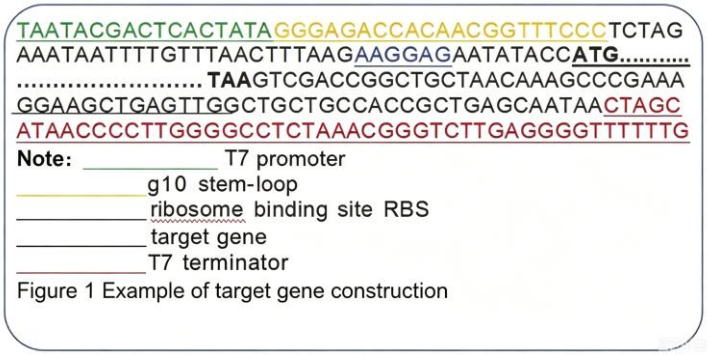
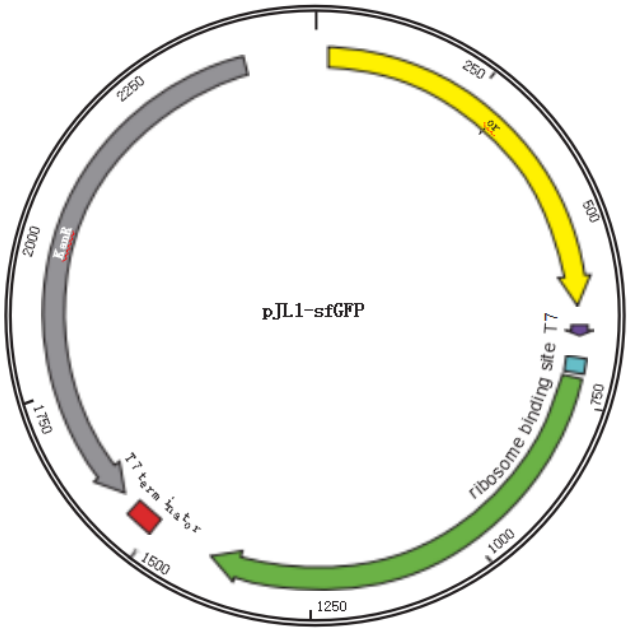


Figure 1 Example of target gene construction

Note: Plasmids containing the lac operator (such as pET-28a) may significantly reduce protein yield and are not recommended for direct use.

Figure 2 Schematic diagram of positive control plasmid DNA sequence



2. Template Preparation

The Cell-Free Protein Synthesis Kit supports the use of DNA or mRNA as templates for recombinant protein expression. DNA templates may include plasmids, PCR products, or RCA products amplified by phi29 polymerase.

(1) Plasmid DNA: Obtain a suitable plasmid via gene synthesis or subcloning, and purify using column-based plasmid extraction kits.

(2) PCR product: Design primers with the forward primer approximately 200 bp upstream of the T7 promoter and the reverse primer approximately 200 bp downstream of the stop codon (including the T7 terminator). Amplify the template; the resulting linear DNA fragment can be used directly in the cell-free reaction without purification. The 200 bp flanking sequences protect linear DNA from degradation by endogenous exonucleases.

(3) RCA product: Perform rolling circle amplification (RCA) using phi29 polymerase and random hexamer primers. The amplified DNA product can be used directly in the cell-free reaction.

(4) PCR and RCA can be combined with Golden Gate and Gibson Assembly to greatly accelerate and increase the throughput of DNA template preparation.

(5) DNA templates must be accurately quantified before use. High-quality plasmid extraction kits are recommended to avoid RNase A contamination. Plasmids received from gene synthesis companies must be column-purified; otherwise, they cannot be used directly in cell-free reactions.

3. Cell-Free Protein Expression

(1) Calculate the required volumes of Solution A and Solution B (volume ratio=1:2) based on total reaction volume. Add all reagents to the reaction vessel (e.g., 2 mL round-bottom centrifuge tube) on

ice and mix gently. Wear gloves and a mask throughout the procedure, and use nuclease-free pipette tips and reaction vessels to avoid nuclease contamination. The reaction system can be prepared according to the table below:

Component	Final Concentration	50 µL System	100 µL System
Cell-free system Solution A Max	30%	15 µL	30 µL
Cell-free system Solution B	60%	30 µL	60 µL
Template DNA	5–10 µg/mL	5–10 µg/mL	5–10 µg/mL
Nuclease-free water	/	to 50 µL	to 100 µL

(2) Add template DNA to the reaction mixture. The recommended final concentration is 5–10 µg/mL; the amount can be further optimized.

(3) Incubate the reaction vessel in a shaking incubator or thermomixer for cell-free protein expression. The recommended reaction temperature is 25-30 °C. Lower temperatures reduce the protein synthesis rate but improve protein solubility. Maximum yield is typically achieved after approximately 8 hours; overnight incubation (16 h) is also applicable. Prolong the reaction time appropriately when using lower temperatures.

(4) Cell-free protein expression requires sufficient oxygen. When using a 2 mL round-bottom centrifuge tube, the reaction volume should not exceed 100 µL. The reaction can be scaled up proportionally using larger vessels (e.g., shake flasks) with a shaking speed of 200 rpm.

4. Detection

After completion of the reaction, take approximately 1 µL of the reaction mixture (for total protein) or supernatant (for soluble protein) and analyze target protein expression by SDS-PAGE.

5. Positive Control

The kit includes a sf-GFP (super-folder GFP) plasmid as a positive control, allowing direct visual observation of results. Upon successful expression of sf-GFP, the cell-free reaction mixture will show a distinct green color. For precise quantification of sf-GFP, detection can be performed using a microplate reader (Ex/Em=485 nm/528 nm).

Contents & Storage

Item No.	Appearance	Components	20 reactions	100 reactions	Storage

Item No.	Appearance	Components	20 reactions	100 reactions	Storage
C1522808A	Liquid	Cell-free system solution A Max	300 µL	2×750 µL	-80°C. Avoid freeze/thaw cycle.
C1522808B	Liquid	Cell-free system solution B	600 µL	3×1 mL	-80°C. Avoid freeze/thaw cycle.
C1522808C	Liquid	CFPS-Control Plasmid	2 µg	2 µg	-80°C. Avoid freeze/thaw cycle.

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Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
- Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the kit for their specific application.

- Aladdin product labels, SDS, COA, and approved specifications take precedence over this document. If product formulation, label, SDS, storage conditions, pack size, or quality specifications change, this document would be reviewed and reissued.