Cell-free Protein Synthesis (*E. coli*) Kit

Item No. 41534

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, remove components and store as stated below.

Item Number	Item	Size
400792	S30 Synthesis Extract	70 µl
400791	Protein Synthesis Buffer (4X)	150 µl
400793	Control His-sfGFP Plasmid	5 µl

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Linear or circular plasmid containing DNA or PCR products for the protein-of-interest (POI)
2. Nuclease-free 1.5 ml microcentrifuge tubes
3. Nuclease-free pipette tips
4. Nuclease-free water and double-distilled water (ddH_2O)
5. Various buffers (loading, lysis/binding, elution, or wash). Buffer selection based on protocol used.
6. DNA exonuclease inhibitor (optional)

INTRODUCTION

About This Assay

Cell-free protein synthesis (CFPS) is an easy-to-use, rapid, and efficient system that is devoid of membrane-bound barriers but contains the native cellular transcriptional and translational machinery and exogenous resources (*e.g.*, amino acids, nucleotides, and a secondary energy substrate) needed for synthesis of the desired proteins.[1-5]

The CFPS (*E. coli*) Kit can produce high levels and different molecular weights of recombinant proteins within several hours and can be used in high-throughput functional genomics and proteomics. In addition, it can provide proteins to drive biochemical and structural biology research.

The synthesized protein can be detected by SDS-PAGE, Western blot, and fluorescence-detection size exclusion chromatography (FSEC) and can be isolated from the reaction mixture by affinity purification techniques for further structural and/or functional characterization.

The CFPS (*E. coli*) Kit is for 10 reactions and can produce high levels and different molecular weights of recombinant protein within several hours and can be used in high-throughput functional genomics and proteomics. In addition, it can provide proteins to drive biochemical and structural biology research.

TEMPLATE PREPARATION

Preparation

A linear or circular plasmid containing DNA or PCR products for the POI can be used as a template. Circular plasmid DNA can generate relatively higher yields, but it is more convenient to use PCR products when performing high-throughout screening. To achieve optimal protein yield, it is important to use high purity and a high concentration of plasmid DNA templates and to avoid adding high concentrations of salts or glycerol with the DNA template. Furthermore, it is recommended to add the appropriate nuclease inhibitors in the Protein Synthesis Buffer to inhibit any contaminating RNase/DNase and to stabilize linear DNA. For convenience, the Control His-sfGFP Plasmid (Item No. 400793) can be used as a cloning vector (Figure 1). This high-copy vector contains the required T7 promoter, ribosome-binding site (RBS), and T7 terminator elements. The sfGFP gene can be replaced with other genes-of-interest for use with *in vitro* expression.

Circular Plasmid DNA as a Template

Plasmids used with this platform must include the following elements and structural features:

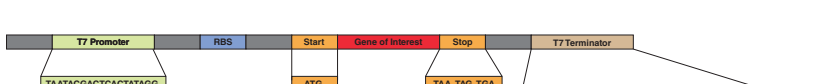


Figure 1. Schematic of the Control His-sfGFP Plasmid

- The target gene must be under control of the T7 promoter that is located upstream from an RBS.
- The distance between the T7 promoter and ATG start codon should be around 20 to 100 nucleotides.
- The T7 terminator must be downstream from the stop codon.

PCR Products as a Template for High-Throughput Screening

For high-throughput screening, PCR products can be used as templates and must contain the required T7 promoter, RBS, and T7 terminator elements. In order to identify high-yield and soluble variants of the POI, different fusion tags can be added at the N- or C- terminus of the POI and expression cassettes can be generated by PC (Figure 2, below).

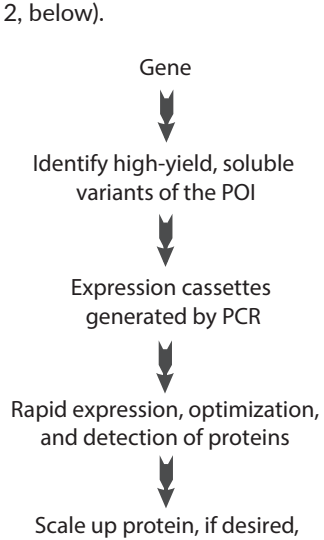


Figure 2. Procedure for high-throughput screening of fusion protein constructs

PROTOCOLS

Cell-free *E. coli* Protein Synthesis Reaction Protocol

The reaction can typically be performed with 25 µl volumes in nuclease-free 1.5 ml tubes for verification of the target protein from a particular DNA sequence. It can also be performed with smaller volumes for high-throughput screening in a 96-well plate format. Reactions can be scaled up to 1 ml as needed. It is necessary to wear gloves and use nuclease-free tubes and tips to prevent contamination. Keep all reagents on ice before and during the assembly of reactions. Avoid repeated freeze-thaw cycles of reagents.

Standard Protocol

1. Thaw all components on ice.
2. Gently vortex the S30 Synthesis Extract (Item No. 400792) and Protein Synthesis Buffer (4X) (Item No. 400791) to mix.
3. Combine reagents in a nuclease-free 1.5 ml tube on ice as follows:

Component	Negative Control	Positive Control	Sample
S30 Synthesis Extract	6 µl	6 µl	6 µl
Protein Synthesis Buffer (4X)	11.5 µl	11.5 µl	11.5 µl
Control His-sfGFP Plasmid	--	250 ng	--
Plasmid of POI	--	--	250 ng
Nuclease-free Water	Up to 25 µl	Up to 25 µl	Up to 25 µl

4. Incubate the reactions at 37°C with vigorous shaking for 2-4 hours
5. Analyze by SDS-PAGE, FSEC, and/or Western blot, or freeze at -20°C for later use

SDS-PAGE Protocol

The synthesis reaction produced by the CFPS (*E. coli*) Kit can be directly loaded onto an SDS-PAGE gel.

1. Combine 5 µl of the synthesis reaction with 5 µl of 2X SDS loading buffer and heat at 100°C for 3-5 minutes.
2. Mix samples evenly and load samples directly onto the gel.
3. Run the gel according to the manufacturer's recommendations.
4. Stain with Coomassie blue or proceed to Western blot.

After staining, the target protein is typically observed as a unique band, which is absent in the negative control reaction. However, sometimes the target has the same apparent molecular weight as an endogenous protein. In this case, the target protein can be detected by Western blot or FSEC.

FSEC Protocol

The target protein produced by the CFPS (*E. coli*) Kit can also be detected by FSEC.

1. Centrifuge 30 µl of the synthesis reaction at 12,000 rpm in a 4°C centrifuge for 1 hour.
2. Combine 30 µl of the synthesis reaction with 30 µl of 2 µM HIS Lite™ Tris NTA-Ni Complex.
3. Incubate at 4°C for 30 minutes.
4. Run FSEC according to the manufacturer's recommendations.

During FSEC, the target protein is typically observed as a unique peak. However, sometimes the target has the same apparent molecular weight as an endogenous protein. In this case, the target protein can be detected by Western blot.

Protein Purification Protocol

Several types of affinity purification methods can be applied. If the synthesized protein contains a His tag, use a Ni-NTA resin, such as His FF. If the synthesized protein has a Strep tag, use Strep resin, as described in the **Affinity Purification Quick Protocol** below.

Affinity Purification Quick Protocol

1. Wash 20 µl beads (His FF/Strep-Tactin® XT) with 1 ml ddH_2O twice.
2. Add 1 ml of lysis/binding buffer to the beads. Centrifuge at 12,000 rpm at 4°C for 2 minutes, then discard the lysis/binding buffer. Repeat three times.
3. Centrifuge 500 µl of the synthesis reaction at 12,000 rpm in a 4°C centrifuge for 1 hour, then add the supernatant of the protein synthesis reaction to the beads, tap to mix, and allow binding for 2-60 minutes. Allow binding to continue for an additional 60 minutes for Strep-Tactin® XT or overnight for His FF.
4. Centrifuge the beads at 12,000 rpm at 4°C for 10 minutes and retain the supernatant.
5. Add 1 ml of wash buffer to the beads and centrifuge at 12,000 rpm at 4°C for 2 minutes. Repeat this three times, retaining the washes.
6. Add 100 µl of elution buffer to the beads. Tap the column repeatedly to mix. Centrifuge at 12,000 rpm at 4°C for 2 minutes, and retain the elution.

ANALYSIS

Analysis of Synthesized Protein

Following synthesis, the samples can be analyzed by SDS-PAGE, ProteinSimple™, Western blot, or FSEC. SDS-PAGE and FSEC protocols are provided on page 11. To verify the effectiveness of the CFPS (*E. coli*) Kit it is highly recommended to analyze synthesis reactions side-by-side with a positive control sample. To compare the banding pattern, it is highly recommended to analyze synthesis reactions side-by-side with a negative control sample.

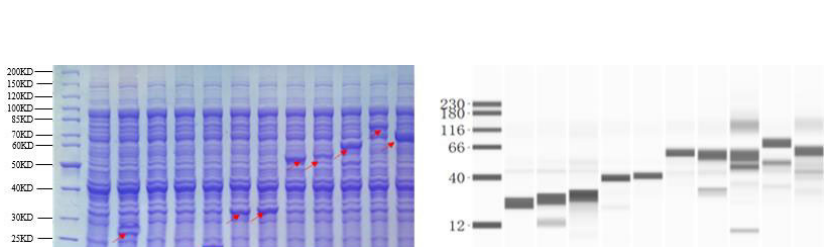


Figure 3. Protein expression using the CFPS (*E. coli*) Kit. 3 µl of each reaction were analyzed by SDS-PAGE using a 4-20% Tris-glycine gel and imaged using ProteinSimple™. Red arrows indicate the POI. M: Protein Marker; N: Negative Control, without DNA; P: Positive Control, with sfGFP.

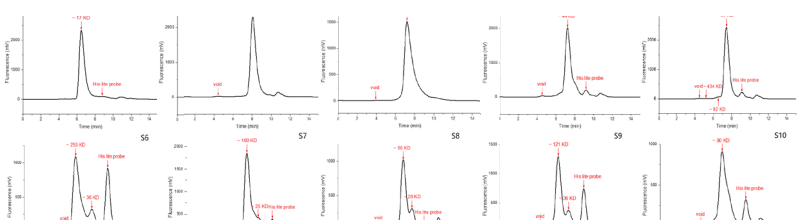
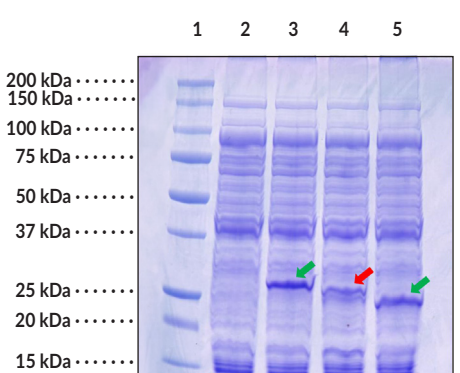


Figure 4. Protein expression using the CFPS (*E. coli*) Kit. 15 µl of each reaction were analyzed by FSEC. The peak marked by red arrows indicates the POI.



Lane 1: MW Markers
Lane 2: Negative Control
Lane 3: sfGFP Positive Control
Lane 4: mCherry
Lane 5: eGFP

Figure 5. SDS-PAGE analysis of protein expression. A 4-20% gradient gel was used to analyze the protein. A 5 µl reaction mixture was mixed with 5 µl of 2X SDS-PAGE loading buffer and heated at 90°C for 10 minutes. 7 µl of the sample was loaded onto the gel. Arrows indicate the target proteins.

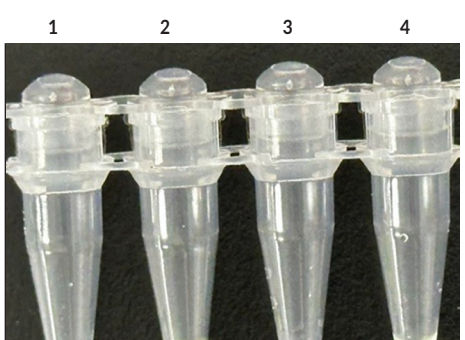


Figure 6. Protein expression using the CFPS (*E. coli*) Kit. Reactions were conducted at 37°C for 3 hours with vigorous shaking. **1**: Negative control; **2**: sfGFP Positive Control; **3**: mCherry; and **4**: eGFP

RESOURCES

References

1. Silverman, A.D., Karim, A.S., and Jewett, M.C. Cell-free gene expression: An expanded repertoire of applications. *Nat. Rev. Genet.* **21(3)**, 151-170 (2020).
2. Garenne, D., Haines, M.C., Romantseva, E., *et al.* Cell-free gene expression. *Nat. Rev.* 1, **49** (2021).
3. Levine, M.Z., Gregprio, N.E., Jewett, M.C., *et al. Escherichia coli*-based cell-free protein synthesis: Protocols for a robust flexible, and accessible platform technology. *J. Vis. Exp.* 144, **e58882** (2019).
4. Jewett, M.C. and Swartz, J.R. Mimicking the *Escherichia coli* cytoplasmic environment activates long-lived and efficient cell-free protein synthesis. *Biotechnol. Bioeng.* **86(1)**, 19-26 (2004).
5. Davanloo, P., Rosenberg, A.H., Dunn, J.J., *et al.* Cloning and expression of the gene for bacteriophage T7 RNA polymerase. *Proc. Nat. Acad. Sci. USA* **81(7)**, 2035-2039 (1984).
6. Dopp, J.L. and Reuel, N.F. Simple, functional, inexpensive cell extract for *in vitro* prototyping of proteins with disulfide bonds. *Biochem. Eng. J.* **164**, 107790 (2020).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.