ZEROTH BIO

Cell-free Protein Synthesis Kit

In vitro transcription & translation — T7 expression system

www.zerothbio.com

Introduction

The Zeroth Bio Cell-free Protein Synthesis Kit enables rapid, efficient *in vitro* transcription and translation directly from template DNA, producing recombinant proteins in an optimized cell-free system. The kit contains a proprietary *E. coli* cell extract and a Master Mix, which are combined with a user-supplied template DNA (plasmid or PCR product) encoding the gene of interest.

After rNTPs and T7 RNA polymerase transcribe the template into mRNA, ribosomes, tRNAs, amino acids, and accessory factors from the optimized *E. coli* extract translate the mRNA into the target recombinant protein. The kit remains fully compatible with the T7 expression system.

Features & Benefits

Feature	Description
Convenient	All components required for transcription and translation are included — simply add template DNA.
Rapid	Synthesize target proteins within 2 or 3 hours, efficiently and reproducibly.
Flexible	Compatible with both circular plasmid DNA and linear PCR products as templates.
Advanced Expression	Enables synthesis of challenging proteins (e.g., cell-toxic, antibodies, membrane, or viral proteins) that are difficult to express in conventional *in vivo* systems.

Kit contents

Component	CFPS kit 100	CFPS kit 200
2x Master Mix	5 vials x 500 µl	5 vials x 1,000 µl
E. coli Extract	5 vials x 300 µl	5 vials x 600 µl

* Note: For research use only. Not for use in diagnostic or therapeutic procedures.

Product Specifications

Specifications	CFPS kit 100	CFPS kit 200
Reactions	50 µl × 100 rxns	50 µl × 200 rxns
Expression System	T7, Batch	
Purification	Not included	
Target Protein Size	≤ 150 kDa	
Protein Yield	0.2 - 2.0 mg/ml	

Storage

Store at −70°C to −20°C. Avoid repeated freeze-thaw cycles. All components must be thawed on ice immediately before use.

Upon receipt, we recommend aliquoting the ***E. coli* Extract** to avoid repeated freeze-thaw cycles and storing at −70°C.

Experimental Procedures

Preparation of Template DNA

- A plasmid or linear DNA (PCR product) may be used as template.
- The template must contain: T7 promoter, ribosomal binding site (RBS), T7 terminator.

Thawing Components

1. Thaw 2x Master Mix, *E. coli* Extract on ice.
2. Briefly spin-down the solutions in all tubes and return to ice. Ensure 2x Master Mix and *E. coli* Extract are fully and uniformly resuspended. Do not generate bubbles in the extract.

Preparing the Reaction Mixture

3. Prepare the reaction mixture on ice as shown below:

Component	Negative Control	CFPS 50 µl rxn
2x Master Mix	25 µl	25 µl
E. coli Extract	15 µl	15 µl
DNA	—	Variable
DW	10 µl	Variable
Total Volume	50 µl	50 µl

* Note: DNA can be either plasmid DNA or PCR-amplified DNA, and the concentration can be adjusted according to the user's needs.
A concentration of approximately 5–8 µg/ml is recommended for optimal results.

4. Gently mix the reaction by tapping the tube or pipetting.

Incubation for Protein Expression

5. Incubate the reaction mixture at 30°C for 3 hours or 37°C for 2 hours
6. Briefly centrifuge the completed reaction mixture before proceeding to analysis.

Analysis of Protein Expression

7. Analyze expressed protein by SDS-PAGE, Western blot, or a relevant bioactivity assay.
8. For SDS-PAGE and Western blot analysis, load **2 µl** of the CFPS reaction mixture. For bioactivity assays, it is recommended to assume a target protein concentration of **0.2–2.0 mg/ml** in the CFPS reaction mixture prior to testing.

Ordering Information

Description	Cat. No.
Zeroth Bio Cell-free Protein Synthesis Kit 100 rxn	C1110
Zeroth Bio Cell-free Protein Synthesis Kit 200 rxn	C1120