

AccuRapid™ Cell-Free Protein Expression Kit

[Cat. No.]
K-7250

AccuRapid™ Cell-Free protein expression kit is a High-yield, High-speed, High-throughput protein synthesis kit.

Description

AccuRapid™ Cell-Free Protein Expression kit is designed for *in vitro* transcription and translation of target DNA to protein in a single reaction. This kit contains an optimized *E.coli* extract containing T7 RNA polymerase for transcription and ribosomes for translation. An optimized AccuRapid™ Master mix provides all other required components, including amino acids, rNTPs, and appropriate salts to express high levels of recombinant proteins. This kit contains reagents for 24 reactions, and each reaction can express up to 20 µg of protein in only 3 hours directly from a variety of DNA templates which contains T7 promoter, T7 terminator and RBS (ribosomal binding site). This flexible system expresses up to 300 µg/mL of protein within only 3 hours from the template DNA which contains T7 promoter, T7 terminator and RBS (ribosomal binding site).

Contents

Components	Quantity
AccuRapid™ Master mix	170 µL × 3 tubes
AccuRapid™ <i>E.coli</i> extract	100 µL × 3 tubes
Positive control DNA	24 µL × 1 tube
DEPC DW	1 mL × 1 tube

Features and Benefits

High-speed

The AccuRapid™ Cell-Free Protein Expression kit can synthesize your recombinant protein in 3 hours

Simple

The AccuRapid™ Cell-Free Protein Expression kit includes all necessary components for transcription and translation; The AccuRapid™ Cell-Free Protein Expression kit is ready to use, and easy to set up.

Flexible

The AccuRapid™ Cell-Free Protein Expression kit can synthesize proteins from various DNA templates. The DNA must include both T7 promoter/terminator site and RBS.

- Note 1) We recommend cloning the template DNA into our *in vitro* transcription/translation optimized pBIVT vector set (sold separately).
- Note 2) Sometimes pET vector series such as pET15b, 22b, 23a, and 28b can also be used for the kit, though this should be tested. In addition PCR products containing T7 promoter, ribosomal binding site, T7 terminator, and His-tag encoding sequences at N- or C-terminus can be used for protein synthesis as a template DNA.
- Note 3) The DNA template for protein expression can be synthesized through our **Gene Synthesis Service**, which optimizes the codons for *E.coli* protein expression. The synthesized gene can be cloned into our *in vitro* transcription/translation vector by request. Please refer to our homepage (www.bioneer.co.kr) for additional information.

Storage

The kit components should be stored at 2 different temperatures: AccuRapid™ *E.coli* extract must be stored below -70 °C; the remaining components should be stored at -20 °C.

Experimental Protocol

1. Thaw the AccuRapid™ Cell-Free Protein Expression kit components on ice.
2. After thawing, spin-down the the AccuRapid™ Master mix and AccuRapid™ *E.coli* extract and place the tube on ice bath.
3. Prepare the reaction mixture (one reaction).

Components	Negative	Positive	Test reaction
AccuRapid™ Master mix	20.8 µL	20.8 µL	20.8 µL
AccuRapid™ <i>E.coli</i> extract	12 µL	12 µL	12 µL
DNA template	-	1 µL	X µL (60 ng - 600 ng)
DEPC-DW	12.2 µL	11.2 µL	(12.2 - X) µL
Total	45 µL	45 µL	45 µL

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- Total volume should be 45 uL in 1.5 mL microcentrifuge tube. Mix reaction mixture by tapping the tube gently and spin down briefly to collect the mixture at the bottom of the tube.
- Incubate the reaction mixture at 30 °C in a water bath for 3 hours.
- After completing incubation, spin down briefly to collect the mixture at the bottom of the tube and place the tube on ice. (The reaction mixture should be stored below -70 °C for future use)
- Analyze the mixture using SDS-PAGE, western blotting, or bioactivity assay.

Analyzing samples (SDS-PAGE Method)

After expression of proteins using *AccuRapid™* Cell-Free Protein Expression Kit, you may use SDS-PAGE to determine the success of expression.

- Prepare loading sample.
; Expression sample, 5 uL + Sterile DW, 10 uL + 4x Loading dye, 5 uL (Final volume ; 20uL)
- Denature all samples at 95°C for 5 min.
- Load 5 uL/well of SDS-PAGE minigel.
- Run X% SDS-PAGE gel electrophoresis.
- Perform staining with coomassie blue R-250.

Ex) Result of positive control (23kDa)
- 12% SDS-PAGE gel, 50V, 20min → 150V, 50min

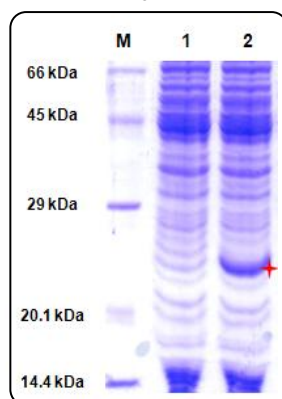


Figure 1: Protein expression Data of *AccuRapid™* Cell-Free Protein Expression Kit (SDS-PAGE and Coomassie Brilliant Blue staining). Lane M; *AccuLadder™* Protein Size Marker (Low), Lane 1; Negative control (No DNA), Lane 2 ; Positive control (CAT : Chloramphenicol acetyl transferase)

Related products

Cat. No.	Products	Size
D-2010	<i>AccuLadder™</i> Protein Size Marker (Broad)	500 uL
D-2020	<i>AccuLadder™</i> Protein Size Marker (Low)	500 uL
K-3030	<i>AccuPrep®</i> Plasmid Mini Extraction Kit	200 preps
K-7300	<i>ExiProgen™</i> EC1 Protein Synthesis Kit	16 reactions
K-7301	<i>ExiProgen™</i> EC1 Protein Synthesis Kit	32 reactions
K-7302	<i>ExiProgen™</i> EC1 Protein Synthesis Kit	96 reactions
K-7350	pBIVT vector set-1	Each 5 ug
A-5041	<i>ExiProgen™</i>	-
S-2041	Gene Synthesis Services	-