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Bioneer R&D Center

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AccuRapid™ Protein Expression Kit is available for a High-yield, High-speed, High-throughput protein expression. [Cat. # K-7250~7270]

1. Description

AccuRapid™ Protein Expression Kit is designed for *in vitro* transcription and translation of target DNA to protein in a single reaction. This product contains an optimized *E.coli* extract containing T7 RNA polymerase for transcription and ribosome for translation. An optimized Master mix provides all other required components, including amino acids, rNTPs, and appropriate salts to express high-level production of recombinant proteins. This product contains reagents for expression of protein directly from a variety of DNA templates, including T7 promoter and terminator as well as RBS (ribosomal binding site). This product can be used to express a wide range of target proteins and has the capacity for a yield of more than 300 µg/mL of protein within 3 hours.

2. Contents

		AccuRapid™ Cell-Free Protein Expression Kit	AccuRapid™ Midi Protein Expression Kit	AccuRapid™ Maxi Protein Expression Kit
Cat. No.		K-7250	K-7260	K-7270
Reactions		45 µL x 24 reactions	1 mL x 5 reactions	10 mL x 1 reaction
Component	Master mix	0.54 mL x 1 tube	1.2 mL x 2 tubes	4.8 mL x 1 bottle
	<i>E.coli</i> extract	0.3 mL x 1 tube	0.28 mL x 5 tubes	2.8 mL x 1 bottle
	DEPC DW	1 mL x 1 tube	1 mL x 2 tubes	3 mL x 1 bottle
	Positive Control DNA	1 tube	1 tube	1 tube

**** storage temperature : -20°C ~ -70°C**

3. Features and Benefits

- ▶ **High-speed:** synthesize your target protein quickly (within 3 hours) and economically
- ▶ **Easy-to-Use:** includes all necessary components for transcription and translation
- ▶ **Flexible:** synthesize proteins from various DNA templates.
 - synthesize proteins of toxic proteins
 - incorporate non-natural amino acids
 - use additives such as metal ions, detergents, cofactors, binding partners

4. Note

1. We recommend cloning the template DNA into our *in vitro* transcription/translation optimized pBIVT vector set (sold separately).
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 proteins synthesis as a template DNA.
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.

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5. Experimental Protocol

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

Components	Negative	Positive	Sample		
			45 µL reaction	1 mL reaction	10 mL reaction
Master mix	21 µL	21 µL	21 µL	470 µL	4.7 mL
<i>E.coli</i> extract	12 µL	12 µL	12 µL	270 µL	2.7 mL
DNA template	-	1 µL	X µL	X µL	X mL
DEPC DW	12 µL	11 µL	(12 - X) µL	(260 - X) µL	(2.6 - X) mL
Total	45 µL	45 µL	45 µL	1000 µL	10 mL

4. Mix reaction mixture by tapping the tube gently and spin down briefly to collect the mixture at the bottom of the tube.
5. Incubate the reaction mixture at 30°C in a water bath for 3 hours.
6. After completing incubation, spin down briefly to collect the mixture at the bottom of the tube.
7. Analyze the mixture using SDS-PAGE, western blotting or bioactivity assay.

6. Analysis of expressed samples

After expression of proteins using *AccuRapid™* Protein Expression Kit, you can the expression of target protein via SDS-PAGE

1. Prepare loading samples.
5 µL of expression sample + 10 µL of D.W. + 5 µL of 4x Loading dye (Final volume is 20 µL)
2. Denature all samples at 95°C for 5 min.
3. Load 5 µL of each samples on SDS-PAGE minigel.
4. Run SDS-PAGE gel.
5. Perform staining with coomassie blue R-250.
Ex) Result of positive control (28kDa)
- condition : 12% SDS-PAGE gel, at 50 V for 20 min → at 150 V for 50 min

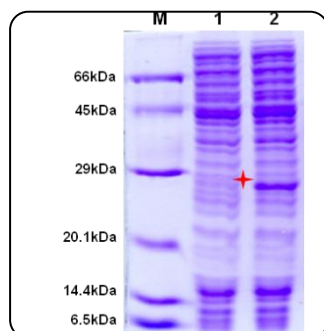


Figure 1: Protein expression Data of *AccuRapid™* Protein Expression Kit (SDS-PAGE and Coomassie Brilliant Blue staining).

Lane M; *AccuLadder™* Protein Size Marker (Low, *Bioneer*, Cat. # D-2020)

Lane 1; Negative control (No DNA),

Lane 2; Positive control (AcGFP: *Aequore coerulescens* GFP fluorescent protein)