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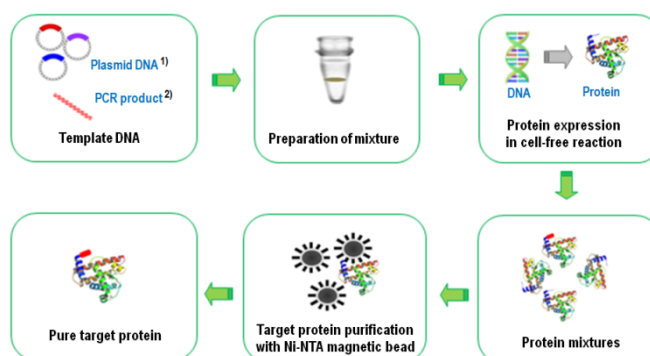
AccuRapid™ Protein Synthesis Kit is available for a High-speed, Super-easy protein synthesis without cell culture. This kit is compatible with ExiProgen™ EC Protein Synthesis Kit. [Cat. # K-7280]

1. Overview

E. coli cell-free protein expression method is the coupled reaction of transcription and translation. The method use a mixture containing template DNA bearing a gene of interest (either expression vector or PCR product), *E. coli* cell extract and other required reagents such as amino acids and rNTPs. rNTPs, and T7 RNA polymerase synthesize mRNA from template DNA, and then ribosome and tRNA are required for the translation step.

Ni-NTA magnetic beads have many advantages over existing resins and are suited for purification of His-tagged recombinant protein. Ni-NTA groups coated on surface of the beads can interact with His-tag of expressed protein to make that protein to bind them on the bead. After washing out unbound proteins, the target proteins can be purified through elution process.

The procedure of protein expression and purification using AccuRapid™ Protein Synthesis Kit is shown below.



¹⁾ A gene of interest should be cloned into a protein Expression vector with T7 expression system and 6X His-tag, such as pBIVT (Cat. # K-7350), pET, pDuet, or pIVEX.

²⁾ ExiProgen™ ProXpress PCR template kit (Cat. # K-7400) can be used to produce a template DNA without cloning.

2. Kit contents and storage

This kit consists of two boxes (Kit ① and Kit ②). The Kit ① and ② contains components for Ni-affinity purification and for *E. coli* cell-free protein expression, respectively.

	Components	Quantity	Storage temperature
Kit ①	Ni-NTA magnetic bead	50 mg x 5 tubes	4 ~ 8℃
	Elution buffer	1.25 mL x 1 tube	
	Binding/washing buffer	25 mL x 1 bottle	
Kit ②	<i>E. coli</i> extract	200 uL x 5 tubes	-20 ~ -70℃
	Master mix	350 uL x 5 tubes	
	DEPC DW	1.0 mL x 1 tube	
	Positive Control DNA	10 uL x 1 tube	

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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

- 4.1. We recommend cloning the template DNA into our *in vitro* transcription/translation optimized pBIVT vector set (sold separately).
- 4.2. 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.
- 4.3. 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.

5. Experimental Protocol

5.1. Expression of proteins with *E. coli* cell-free protein expression method

- 1) Take out the *E. coli* extract, Master mix, and DEPC DW from Kit ② and thaw them on ice.
- 2) When using Positive Control DNA, take out the Positive Control DNA tube from the Kit ② and thaw it on ice.
note) The pBIVT-AcGFP of about 3.8 kb size, which corresponds to 28 kDa protein is provided as a Positive Control DNA.
- 3) Prepare protein expression mixture in 1.5 mL microcentrifuge tube using pre-thawed *E. coli* extract, Master mix and DEPC DW. (It is necessary to spin-down each tube at a microcentrifuge followed by mixing through a pipette before use.)

	Negative Control	Positive Control	Sample
Template DNA	-	10 µL	X µL
<i>E. coli</i> extract	200 µL	200 µL	200 µL
Master mix	350 µL	350 µL	350 µL
DEPC DW	200 µL	190 µL	(200-X) µL
Total	750 µL	750 µL	750 µL

Note) The amount of template DNA, in microgram quantity, can be determined as follows. For plasmid DNA, use 1 µg per kb (kilobase) of that DNA, in proportional to template DNA size. For PCR product, use 500 ng (for amplicons less than 1 kb in size), or 1 µg (for amplicons of 1~2 kb). The template DNA should have $A_{260/280} > 1.8$ and $A_{260/230} > 1.5$ for optimal protein expression.
If you want to get the maximum amount of protein expressed, please refer to the Appendix for further information.

- 4) Mix the mixture by pipetting gently and incubate at 30°C for 3 hours using water bath or heating block.
- 5) After incubation, spin down the mixture briefly to collect all components at bottom of the tube.
- 6) Take 10 µL sample of the supernatant for SDS-PAGE analysis. (* Supernatant from this step is Expression sample.)

5.2. Purification of target protein with Ni-affinity purification method

Take out the Ni-NTA magnetic bead, Binding/washing buffer, and Elution buffer from Kit ①.

A. Purification protocol using vortexer and microcentrifuge

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- 1) Pipette 500 µL of the magnetic bead solution from Ni-NTA magnetic bead tube and transfer it into a 1.5 mL tube.
- 2) Spin-down and remove supernatant from magnetic bead.
- 3) Add 1.0 mL of Binding/washing buffer to the bead, and then resuspend the bead by vortexing.
- 4) Spin-down and remove supernatant from the bead.
- 5) Add the protein expression sample (about 700 µL) to the bead, and then mix by vortexing.
- 6) Incubate at room temperature for 5 min.
- 7) Spin-down and remove supernatant from the bead. (* Supernatant from this step is Unbound sample.)
- 8) Add 1.0mL of Binding/washing buffer to the bead and wash the bead by vortexing.
- 9) Spin-down and remove supernatant from the bead. (* Supernatant from this step is Washing sample.)
- 10) Repeat step 8) and 9) three times.
- 11) Add 0.25 mL of Elution buffer to the bead and elute target proteins from the bead by vortexing.
- 12) Incubate at room temperature for 5 min.
- 13) Spin-down and transfer supernatant to a new 1.5 mL microcentrifuge tube.
(* Supernatant from this step is Elution sample.)
- 14) Analyze the supernatant using SDS-PAGE, western blotting, or bioactivity assay.

B. Purification protocol using *MagListo*™-2 Magnetic Separation Rack (Cat. No. TM-1010)

- 1) Place the Ni-NTA magnetic bead tube in the separation rack of *MagListo*™-2 without magnet plate.
- 2) Combine magnet plate with separation rack and remove supernatant from the bead.
- 3) After separating the magnet plate from separation rack, add 1.0 mL of Binding/washing buffer to the bead and resuspend the bead by pipetting.
- 4) Combine magnet plate with separation rack and remove Binding/washing buffer from the bead.
- 5) After separating magnet plate from separation rack, add the protein expression sample (about 700 µL) to the bead.
- 6) Mix protein expression mixture and magnetic bead by pipetting and incubate at room temperature for 5 min.
- 7) Combine magnet plate with separation rack and remove supernatant from the bead.
(* Supernatant from this step is Unbound sample.)
- 8) After separating magnet plate from separation rack, add 1.0mL of Binding/washing buffer to magnetic bead and wash the bead by pipetting.
- 9) Combine magnet plate with separation rack and remove supernatant from the bead.
(* Supernatant from this step is Washing sample.)
- 10) Repeat step 8) and 9) three times.
- 11) After separating magnet plate from separation rack, add 0.25 mL of Elution buffer to the bead and elute target proteins from the bead by pipetting.
- 12) Incubate at room temperature for 5 min.
- 13) Combine magnet plate with separation rack and transfer supernatant to a new 1.5 mL microcentrifuge tube.
(* Supernatant from this step is Elution sample.)
- 14) Analyze the supernatant using SDS-PAGE, western blotting, or bioactivity assay.

4. Analysis of expressed samples

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

- 1) Prepare loading mixture as shown in the table.

	Expression (Unbound & Washing) sample	Elution sample
Sample	5 µL	15 µL
4x loading dye	5 µL	5 µL
DW	10 µL	-
Total	20 µL	20 µL

- 2) Treat the mixtures at 95 °C for 5-10 min.
- 3) Prepare SDS-PAGE mini-gel with about 10% ~ 12%.
- 4) Load **5 µL each of "Expression, Unbound and Washing samples" and 10 µL of "Elution sample"** to each well and run the SDS-PAGE.
- 5) After staining the gel with Coomassie blue solution, a band of target protein can be detected as below.

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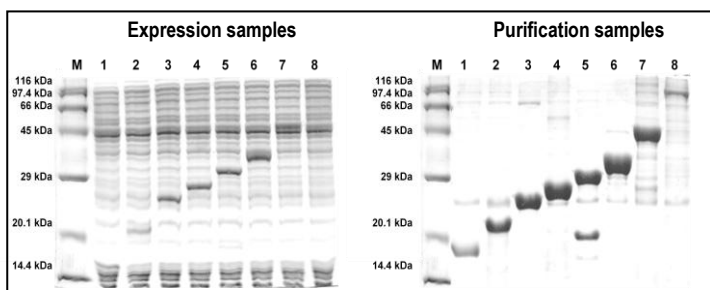


Fig. 1. The expression and purification of target protein with wide range M.W. using *AccuRapid™* Protein Synthesis Kit M; *AccuLadder™* Protein Size Marker (Broad, Bioneer, Cat. # D-2010), 1; CalmL3 (17.5kDa), 2; DUSP 3 (22kDa), 3; CAT (24kDa), 4; AcGFP (29kDa), 5; ERFP (31kDa), 6; EF-Ts (34kDa), 7; VF (45kDa), 8; BM3 (117kDa)

5. Appendix : Determination of DNA template concentration

A different amount of DNA template in protein synthesis reaction may result in a different yield of target protein. It is strongly recommended to optimize the amount (or concentration) of template DNA for protein synthesis. For the optimization of template DNA amount it is useful to use the *AccuRapid™* Cell-free Protein Expression Kit (Cat. # K-7250). Protocol is shown in the below.

AccuRapid™ Cell-Free Protein Expression Kit (Cat. # K-7250)

1. Prepare template DNA with the following concentrations.
Ex) 10, 20, 40, 60, 80, and 100 (ng/uL)
2. Express the target protein following the manual of *AccuRapid™* Cell-free Protein Expression Kit
 - 1) Prepare reaction mixture
 - Reaction volume ; 15 uL
 - Sample DNA ; Add 2 uL of template DNA from #1.
(Final DNA amount - 20, 40, 80, 120, 160, and 200 ng/rxn)
 - 2) Incubate the mixture at 30 °C for 3 hrs with PCR machine.
 - 3) Verify the amount of template DNA which shows the highest yield of target protein via SDS-PAGE.



AccuRapid™ Protein Synthesis Kit

3. Determine the **50-times** as much DNA as the optimized amount from 2.
4. Add the determined amount of template DNA from 3. Then synthesize protein using the *AccuRapid™* Protein Synthesis Kit.

6. Related Products

Cat. No.	Product	Size
K-7250	<i>AccuRapid™</i> Cell-Free Protein Expression Kit	45 uL x 24 reactions
K-7300~7302	<i>ExiProgen™</i> EC Protein Synthesis Kit	16/32/96 reactions
K-7350	pBIVT vector set-1	Each 5 ug
K-7400~7401	<i>ExiProgen™</i> ProXpress PCR Template Kit	16/32 reactions
D-2010	<i>AccuLadder™</i> Protein Size Marker (Broad)	500uL
D-2020	<i>AccuLadder™</i> Protein Size Marker (Low)	500uL
TM-1010	<i>MagListo™-2</i> Magnetic Separation Rack	-
A-5041	<i>ExiProgen™</i>	-