AccuRapid™ Protein Synthesis Kit

[Cat. No.] K-7280

Introduction

AccuRapid™ Protein Synthesis Kit allows an expression and a purification of target proteins without cell culture. This product contains all the components required for cell-free protein expression and Ni-NTA affinity purification those of His-tagged proteins.
Cell-free protein expression method is a coupled reaction of *in vitro* transcription and translation from target DNA, which produces recombinant proteins in a cell-free system. rNTPs and T7 RNA polymerase are used to synthesize mRNA from a template DNA. And ribosomes, tRNAs, amino acids, and etc. are required for a translation step to synthesize recombinant proteins. These materials are supplied by optimized *E. coli* extract and Master mix in the Kit ①, and these are used with a template DNA bearing a gene of interest (either plasmid or PCR product).
Ni-NTA affinity purification method uses Ni-NTA magnetic beads provided in the Kit ② and are suited for the purification of His-tagged recombinant proteins. Ni-NTA groups coated on the surface of the magnetic beads can interact with His-tag of expressed proteins. After washing out unbound proteins, the target proteins can be purified through an elution process.

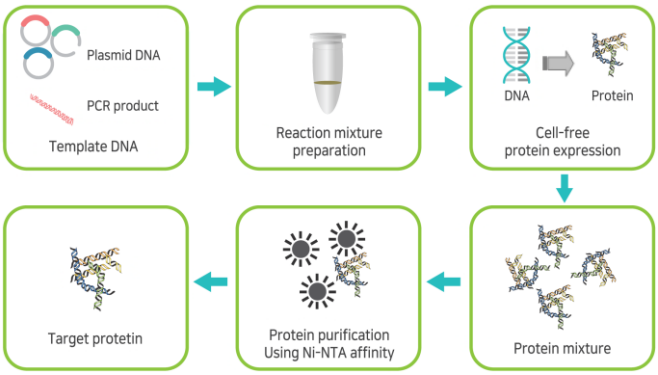


Figure 1. Workflow for protein synthesis

Features & Benefits

- Convenient: Includes all necessary components for protein expression and purification.
- Rapid: Synthesizes target proteins quickly and economically.
- Flexible: Synthesizes proteins from various types of DNA (plasmid or PCR product).
- Advanced expression technologies: Expression of proteins (cell-toxic proteins, antibodies, membrane proteins, viral proteins, etc.) difficult to be done in the existing *in vivo* technology is made possible.

Components

	Components	Amount
Kit ①	Ni-NTA magnetic bead	50 mg x 5 ea
	Elution buffer	1.25 ml x 1 ea
	Binding/washing buffer	25 ml x 1 ea
Kit ②	*E. coli* extract	200 µl x 5 ea
	Master mix	350 µl x 5 ea
	DEPC DW	1.0 ml x 1 ea
	Positive Control DNA	10 µl x 1 ea

*** Note:** The Kit ① and ② contains components for Ni-NTA affinity purification and cell-free protein expression respectively.

Specifications

AccuRapid™ Protein Synthesis Kit	
Reactions	750 µl x 5 rxns
Expression	Yes (T7 system, Batch type)
Purification	Yes
Target protein size	≤ 150 kDa
Protein Yield	≤ 100 µg/rxn

*** Note:** The protein yield can be varied depending on the type of target protein.

Storage

- Store Kit ① at a temperature between 4°C and 8°C.
- Store Kit ② at a temperature between -70°C and -20°C.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols.

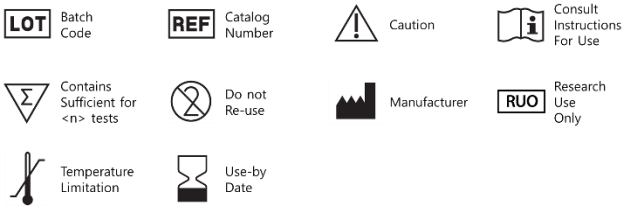
Ordering Information

Description	Cat. No.
AccuRapid™ Protein Synthesis Kit	K-7280

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

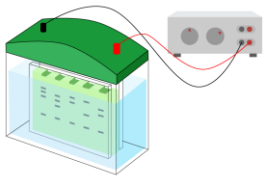
Explanation of Symbols



Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td colspan="3">Preparation of template DNA
• A plasmid or a linear DNA (PCR products) can be used as a template DNA.
• The template DNA must include a T7 promoter, a ribosomal binding site (RBS), a T7 terminator, and a 6x histidine tag at either N- or C-terminal.</td></tr>
<tr><td colspan="3">Cell-free protein expression</td></tr>
<tr><td>1</td><td> 
Thawing materials</td><td>1. Take out the Master mix, E. coli extract, DEPC DW, and Positive Control DNA from Kit ② and thaw them on ice.
* Note: The pBIVT-AcGFP of about 3.8 kb size is provided as a Positive Control DNA, which has a molecular weight of about 28 kDa.
2. Briefly spin them down and then place tubes on ice.
* Note: Make sure that the Master mix and E. coli extract are evenly resuspended before use (Be careful not to create bubbles in the extract).</td></tr>
<tr><td>2</td><td> 
Preparation of protein expression mixture</td><td>3. Prepare the protein expression mixture.
• Preparation of protein expression mixture
<table>
<tr><th>Components</th><th>Negative</th><th>Positive</th><th>Sample</th></tr>
<tr><td>Template DNA</td><td>-</td><td>10 µl</td><td>Variable</td></tr>
<tr><td>E. coli extract</td><td>200 µl</td><td>200 µl</td><td>200 µl</td></tr>
<tr><td>Master mix</td><td>350 µl</td><td>350 µl</td><td>350 µl</td></tr>
<tr><td>DEPC DW</td><td>200 µl</td><td>190 µl</td><td>Variable</td></tr>
<tr><td>Total volume</td><td>750 µl</td><td>750 µl</td><td>750 µl</td></tr>
</table>
* Note: The amount of template DNA can be determined as follows.
For plasmid DNA, use 1 µg per kb of that DNA, in proportion 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}/A_{280}>1.8 and A_{260}/A_{230}>1.5 for optimal protein expression.
4. Gently mix the protein expression mixture by tapping or pipetting.</td></tr>
<tr><td>3</td><td> 
Incubation for protein expression</td><td>5. Incubate the reaction mixture at 30°C for 3 hrs in a water bath or a heat block.
6. Briefly spin down the reaction mixture.
7. Take 10 µl of the supernatant for SDS-PAGE analysis.
* Note: Supernatant from this step is the <u>Expression sample</u> for checking the expression efficiency.</td></tr>
<tr><td colspan="3">Ni-NTA affinity purification of target proteins
• Centrifuge or use magnet for target protein purification.</td></tr>
<tr><td colspan="3">Purification protocol with centrifuge</td></tr>
<tr><td>4</td><td> 
Equilibrating Ni-NTA magnetic bead</td><td>8. Take out the Ni-NTA magnetic bead, Binding/washing buffer, and Elution buffer from Kit ①.
9. Transfer 500 µl of Ni-NTA magnetic bead to a 1.5 ml tube.
10. Briefly spin down and remove the supernatant.
11. Equilibrate by adding 1 ml of Binding/washing buffer and resuspending the Ni-NTA magnetic bead.
12. Centrifuge for 5 sec at 12,000 rpm and remove the supernatant with a pipette.</td></tr>
</table>

5	 **Loading protein expression sample**	13. Load about 700 µl of the protein expression sample onto the pre-equilibrated Ni-NTA magnetic bead and gently mix. 14. Incubate for 5 min at room temperature. 15. Centrifuge for 5 sec at 12,000 rpm and remove the supernatant with a pipette. *** Note:** Supernatant from this step is the **Unbound sample** for checking the binding efficiency.
6	 **Washing magnetic bead**	16. Add 1 ml of Binding/washing buffer and wash the magnetic bead by vortexing or pipetting. 17. Centrifuge for 5 sec at 12,000 rpm and remove the supernatant with a pipette. *** Note:** Supernatant from this step is the **Washing sample** for checking the washing conditions. 18. Repeat step 16 and 17 three times. *** Note:** After the final wash, the remaining Binding/washing buffer should be removed completely.
7	 **Eluting target proteins**	19. Add 250 µl of Elution buffer to elute target proteins from Ni-NTA magnetic bead and gently mix. 20. Incubate for 5 min at room temperature. 21. Centrifuge for 30 sec at 12,000 rpm and collect the supernatant with a pipette. *** Note:** Supernatant from this step is the **Elution sample** for checking the final protein synthesis.
Purification protocol with Neodymium (Nd) magnet • You can also use BIONEER's *MagListo*™-2 Magnetic Separation Rack (not provided, Cat. No. TM-1010) instead of an Nd magnet.		
4	 **Equilibrating Ni-NTA magnetic bead**	8. Take out the Ni-NTA magnetic bead, Binding/washing buffer, and Elution buffer from Kit ①. 9. Transfer 500 µl of Ni-NTA magnetic bead to a 1.5 ml tube. 10. Place the tube on a Nd magnet (not provided) for 1 min and remove the supernatant with a pipette. 11. Remove the tube from the Nd magnet, equilibrate by adding 1 ml of Binding/washing buffer to the bead slurry and mix briefly. 12. Place the tube on the Nd magnet for 1 min and remove the supernatant with a pipette.
5	 **Loading protein expression sample**	13. Remove the tube from the Nd magnet, load about 700 µl of the protein expression sample onto the pre-equilibrated Ni-NTA magnetic bead and gently mix. 14. Incubate for 5 min at room temperature. 15. Place the tube on the Nd magnet for 1 min. 16. Remove the supernatant with a pipette. *** Note:** Supernatant from this step is the **Unbound sample** for checking the binding efficiency.

6	 **Washing magnetic bead**	17. Remove the tube from the Nd magnet, add 1 ml of Binding/washing buffer and wash the magnetic bead by vortexing or pipetting. 18. Place the tube on the Nd magnet for 1 min and remove the supernatant with a pipette. *** Note:** Supernatant from this step is the **Washing sample** for checking the washing conditions. 19. Repeat step 17 and 18 three times. *** Note:** After the final wash, the remaining Binding/washing buffer should be removed completely.
7	 **Eluting target proteins**	20. Remove the tube from the Nd magnet, add 250 µl of Elution buffer to elute target proteins from Ni-NTA magnetic bead and gently vortex. 21. Incubate for 5 min at room temperature. 22. Place the tube on a Nd magnet for 1 min and collect the supernatant with a pipette. *** Note:** Supernatant from this step is the **Elution sample** for checking the final protein synthesis.
Identification of protein synthesis		
8	 **Analysis with SDS-PAGE**	23. Analyze the samples using SDS-PAGE, western blot, or bioactivity assay. • **Protocol for SDS-PAGE analysis.** 1) Prepare the loading mixtures as shown tin the table.

Components	Expression/Unbound/ Washing sample	Elution sample
Sample	5 µl	15 µl
4X Loading dye	5 µl	5 µl
Sterile distilled water	10 µl	-
Total volume	20 µl	20 µl

2) Incubate the samples at 95°C for 5-10 min.

3) Load each sample to the wells of SDS-PAGE gel [10 x 8 (cm), 0.75 mm thick, 10-well].
- Expression, Unbound, and washing samples: 5 µl/well,
- Elution sample: 10 µl/well

4) Perform SDS-PAGE.

5) Stain the gel with Coomassie Blue R-250.