

[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.

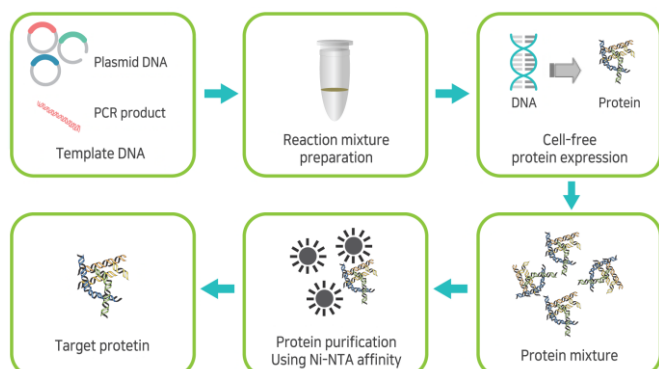


Figure 1. Workflow for protein synthesis

## 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	1.4 ml
	Binding/washing buffer	30 ml
Kit ②	<i>E. coli</i> extract	200 µl x 5 ea
	Master mix	350 µl x 5 ea
	DEPC DW	1.25 ml

Positive Control DNA	12 µl
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\* 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

## 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.

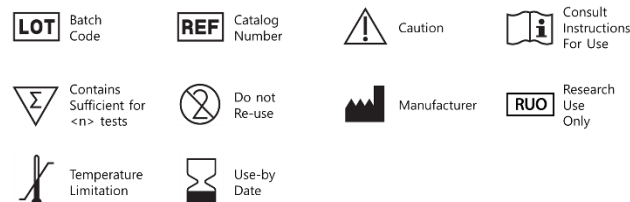
## Ordering Information

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





## Notice

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## Explanation of Symbols




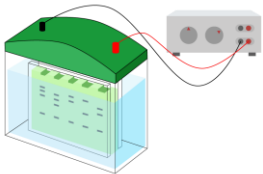


## Experimental Procedures

Steps		Procedure Details																								
<p><b>Preparation of template DNA</b></p> <ul style="list-style-type: none"> <li>A plasmid or a linear DNA (PCR products) can be used as a template DNA.</li> <li>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.</li> </ul>																										
<p><b>Cell-free protein expression</b></p>																										
1	 <p><b>Thawing materials</b></p>	<p>1. Take out the Master mix, <i>E. coli</i> extract, DEPC DW, and Positive Control DNA from Kit ② and thaw them on ice.</p> <p>* <b>Note:</b> 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.</p> <p>2. Briefly spin them down and then place the tube on ice.</p> <p>* <b>Note:</b> Make sure that the Master mix and <i>E. coli</i> extract are evenly resuspended before use (be careful not to create bubbles in the extract).</p>																								
2	 <p><b>Preparation of protein expression mixture</b></p>	<p>3. Prepare the protein expression mixture.</p> <ul style="list-style-type: none"> <li>Preparation of protein expression mixture</li> </ul> <table border="1"> <thead> <tr> <th>Components</th> <th>Negative</th> <th>Positive</th> <th>Sample</th> </tr> </thead> <tbody> <tr> <td>Template DNA</td> <td>-</td> <td>10 µl</td> <td>Variable</td> </tr> <tr> <td><i>E. coli</i> 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> </tbody> </table> <p>* <b>Note:</b> 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 <math>A_{260}/A_{280} &gt; 1.8</math> and <math>A_{260}/A_{230} &gt; 1.5</math> for optimal protein expression.</p> <p>4. Gently mix the protein expression mixture by tapping or pipetting.</p>	Components	Negative	Positive	Sample	Template DNA	-	10 µl	Variable	<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	Variable	Total volume	750 µl	750 µl	750 µl
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3	 <p><b>Incubation for protein expression</b></p>	<p>5. Incubate the reaction mixture at 30°C for 3 hrs in a water bath or a heat block.</p> <p>6. Briefly spin down the reaction mixture.</p> <p>7. Take 10 µl of the supernatant for SDS-PAGE analysis.</p> <p>* <b>Note:</b> Supernatant from this step is the <b>Expression sample</b> for checking the expression efficiency.</p>																								
<p><b>Ni-NTA affinity purification of target proteins</b></p> <ul style="list-style-type: none"> <li>Centrifuge or use magnet for target protein purification.</li> </ul>																										
<p><b>Purification protocol with centrifuge</b></p>																										
4	 <p><b>Equilibrating Ni-NTA magnetic bead</b></p>	<p>8. Take out the Ni-NTA magnetic bead, Binding/washing buffer, and Elution buffer from Kit ①.</p> <p>9. Transfer 500 µl of Ni-NTA magnetic bead to a 1.5 ml tube.</p> <p>10. Briefly spin down and remove the supernatant.</p> <p>11. Equilibrate by adding 1 ml of Binding/washing buffer and resuspending the Ni-NTA magnetic bead.</p> <p>12. Centrifuge for 5 sec at 12,000 rpm and remove the supernatant with a pipette.</p>																								

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5	 <p><b>Loading protein expression sample</b></p>	<p>13. Load about 700 µl of the protein expression sample onto the pre-equilibrated Ni-NTA magnetic bead and gently mix.</p> <p>14. Incubate for 5 min at room temperature.</p> <p>15. Centrifuge for 5 sec at 12,000 rpm and remove the supernatant with a pipette. * <b>Note:</b> Supernatant from this step is the <b>Unbound sample</b> for checking the binding efficiency.</p>
6	 <p><b>Washing magnetic bead</b></p>	<p>16. Add 1 ml of Binding/washing buffer and wash the magnetic bead by vortexing or pipetting.</p> <p>17. Centrifuge for 5 sec at 12,000 rpm and remove the supernatant with a pipette. * <b>Note:</b> Supernatant from this step is the <b>Washing sample</b> for checking the washing conditions.</p> <p>18. Repeat step 16 and 17 three times. * <b>Note:</b> After the final wash, the remaining Binding/washing buffer should be removed completely.</p>
7	 <p><b>Eluting target proteins</b></p>	<p>19. Add 250 µl of Elution buffer to elute target proteins from Ni-NTA magnetic bead and gently mix.</p> <p>20. Incubate for 5 min at room temperature.</p> <p>21. Centrifuge for 30 sec at 12,000 rpm and collect the supernatant with a pipette. * <b>Note:</b> Supernatant from this step is the <b>Elution sample</b> for checking the final protein synthesis.</p>
<p><b>Purification protocol with magnet</b></p>		
4	 <p><b>Equilibrating Ni-NTA magnetic bead</b></p>	<p>8. Take out the Ni-NTA magnetic bead, Binding/washing buffer, and Elution buffer from Kit ①.</p> <p>9. Transfer 500 µl of Ni-NTA magnetic bead to a 1.5 ml tube.</p> <p>10. Place the tube on a Neodymium (Nd) magnet for 1 min and remove the supernatant.</p> <p>11. Remove the tube from the Nd magnet, equilibrate by adding 1 ml of Binding/washing buffer to the bead slurry and mix briefly.</p> <p>12. Place the tube on the Nd magnet for 1 min and remove the supernatant with a pipette.</p>
5	 <p><b>Loading protein expression sample</b></p>	<p>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.</p> <p>14. Place the tube on the Nd magnet for 1 min</p> <p>15. Remove the supernatant with a pipette. * <b>Note:</b> Supernatant from this step is the <b>Unbound sample</b> for checking the binding efficiency.</p>

6	 <p><b>Washing magnetic bead</b></p>	<p>16. Remove the tube from the Nd magnet, add 1 ml of Binding/washing buffer and wash the magnetic bead by vortexing or pipetting.</p> <p>17. Place the tube on the Nd magnet for 1 min and remove the supernatant with a pipette.</p> <p>* <b>Note:</b> Supernatant from this step is the <b>Washing sample</b> for checking the washing conditions.</p> <p>18. Repeat step 16 and 17 three times.</p> <p>* <b>Note:</b> After the final wash, the remaining Binding/washing buffer should be removed completely.</p>															
7	 <p><b>Eluting target proteins</b></p>	<p>19. 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.</p> <p>20. Incubate for 5 min at room temperature.</p> <p>21. Place the tube on a Nd magnet for 1 min and collect the supernatant with a pipette.</p> <p>* <b>Note:</b> Supernatant from this step is the <b>Elution sample</b> for checking the final protein synthesis.</p>															
 <p><b>Option</b></p>		<ul style="list-style-type: none"> <li>You can also use BIONEER's <i>MagListo™-2</i> Magnetic Separation Rack (Cat. No. <b>TM-1010</b>) instead of an Nd magnet.</li> </ul>															
<p><b>Identification of protein synthesis</b></p>																	
8	 <p><b>Analysis with SDS-PAGE</b></p>	<p>22. Analyze the supernatant using SDS-PAGE, western blot, or bioactivity assay.</p> <p>ex) Protocol for SDS-PAGE.</p> <table border="1" data-bbox="598 1189 1182 1391"> <thead> <tr> <th></th> <th>Expression (Unbound/Washing)</th> <th>Elution</th> </tr> </thead> <tbody> <tr> <td>Sample</td> <td>5 µl</td> <td>15 µl</td> </tr> <tr> <td>4X Loading dye</td> <td>5 µl</td> <td>5 µl</td> </tr> <tr> <td>Distilled water (D.W.)</td> <td>10 µl</td> <td>-</td> </tr> <tr> <td>Total volume</td> <td>20 µl</td> <td>20 µl</td> </tr> </tbody> </table> <ul style="list-style-type: none"> <li>- Denaturize at 95°C for 5 min.</li> <li>- Load 5 µl each of "Expression, Unbound and Washing sample" and 10 µl of "Elution sample" on the SDS-PAGE gel.</li> <li>- Run SDS-PAGE.</li> <li>- Stain the gel with Coomassie Blue R-250.</li> </ul>		Expression (Unbound/Washing)	Elution	Sample	5 µl	15 µl	4X Loading dye	5 µl	5 µl	Distilled water (D.W.)	10 µl	-	Total volume	20 µl	20 µl
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