CREATIVE BIOMART®
Assay Kit

Protein Synthesis Kit

Product Information

Cat

Kit-0886

Common Name

Protein

Cat.No.

Kit-0886

Product 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.

Description

Protein Synthesis Kit uses a manual procedure for protein expression and purification. It includes all components required for cell-free protein expression using E. coli extract and Ni-NTA affinity purification. Target protein is recovered in about 100 μg per reaction.

Kit Components

This kit consists of two boxes (Kit□ and Kit□). The Kit□ and Kit□ contains components for Ni-affinity purification and
for E. coli cell-free protein expression, respectively.
Kit□ (store at 4 ~ 8°C):
Ni-NTA magnetic bead: 50 mg x 5 tubes;

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Elution buffer: 1.25 mL x 1 tube;
Binding/washing buffer: 25 mL x 1 bottle.
Kit (store at -20°C ~ - 70°C):
E. coli extract 200 uL × 5 tubes;
Master mix 350 uL × 5 tubes;
DEPC DW 1.0 mL × 1 tube;
Positive Control DNA 10 μL x 1 tube.

Features & 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

Assay Protocol

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 and thaw them on ice.
2) When using Positive Control DNA, take out the Positive Control DNA tube from the Kit 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: E. coli extract 200 μL; Master mix 350 μL; DEPC DW 200 μL; Total 750 μL.
Positive Control: Template DNA 10 μL; E. coli extract 200 μL; Master mix 350 μL; DEPC DW 190 μL; Total 750 μL.
Sample: Template DNA X μL; E. coli extract 200 μL; Master mix 350 μL; DEPC DW (200-X) μL; Total 750 μL.

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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 ug (for amplicons of 1~2 kb). The template DNA should have A260/280 >1.8 and A260/230 >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.

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□.

A. Purification protocol using vortexer and microcentrifuge

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 uL) 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.)

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14) Analyze the supernatant using SDS-PAGE, western blotting, or bioactivity assay.
B. Purification protocol
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 pippetting.
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 ul) 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.

Analysis

After synthesis of proteins using Protein Synthesis Kit, you can the expression of target protein via SDS-PAGE
1) Prepare loading mixture as shown in the table.

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Expression (Unbound & Washing) sample: sample 5μL; 4x loading dye 5μL; DW 10 μL; Total 20 μL.
Elution sample: sample 15μL; 4x loading dye 5μL; Total 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 sample" 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.

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