

Midi Protein Expression Kit

Cat. No. Kit-0884 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Midi 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 g/mL of protein within 3 hours
Applications	This product can be used to express a wide range of target proteins and has the capacity for a yield of more than 300 g/mL of protein within 3 hours.
Storage	Store kit at -20°C ~ -70°C.
Size	1 mL x 5 reactions
Kit Components	Master mix: 1.2 mL × 2 tube; E.coli extract: 0.28 mL × 5 tube; DEPC DW: 1 mL × 2 tube; Positive Control DNA: 1 tube.
Features & Benefits	High-speed: synthesize your target protein quickly (within 3 hours) and economically

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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. Thaw components in the Midi 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).
 Negative: Master mix 21 μ ; E.coli extract 12 μ ; DEPC DW 12 μ ; Total 45 μ .
 Positive: Master mix 21 μ ; E.coli extract 12 μ ; DNA template 1 μ ; DEPC DW 11 μ ; Total 45 μ .
 Sample: Master mix 470 μ ; E.coli extract 270 μ ; DNA template X μ ; DEPC DW (260-X) μ ; Total 1000 μ .
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.

Analysis

- After expression of proteins using Midi Protein Expression Kit, you can the expression of target protein via SDSPAGE
1. Prepare loading samples.
 5 μ of expression sample + 10 μ of D.W. + 5 μ of 4x Loading dye (Final volume is 20 uL)
 2. Denature all samples at 95°C for 5 min.
 3. Load 5 μ 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

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