CREATIVE BIOLABS

Magic™ Mammalian Cell-Free Protein Expression Kit

User Manual

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PRODUCT INFORMATION

Product Overview	Magic™ Mammalian Cell-Free Protein Expression Kit
Product Description	This kit is a unique mammalian cell-free (human cell lysate-based) protein expression systems for *in vitro* translation or coupled transcription/translation reactions. It contains optimized all necessary components for protein synthesis (ribosomes, translation initiation/elongation factors, and tRNA, *etc*.). Modifications such as glycosylation and phosphorylation could be easily obtained. The procedure can be performed in a single reaction tube and is easily scalable without the need for specialized equipment. With results available in a few hours, the kit saves valuable laboratory time and is ideal for high throughput technologies.
Kit Contents (10 x 20-µL reactions)	**Reagents** — **Volume** Cell Extract — 100 µL Reaction Buffer — 60 µL Accessory Proteins — 10 µL Amino Acid Mix — 20 µL T7 RNA Polymerase — 10 µL (200 U/µL) Cloning vector — 10 µg (0.5 µg/µL) Control vector — 1.5 µg (0.3 µg/µL)
Features:	• Protein of interest can be synthesized and visualized in a few hours • Simple protocol and easy setup with all necessary components • Modifications such as glycosylation and phosphorylation could be easily obtained • Protein can be reverse-purified or subject to downstream applications • High throughput screening, synthetic biology, toxic or difficult to express protein synthesis, studies on protein folding, activity and protein-protein interactions

General protocol

The Magic™ Mammalian Cell-Free Protein Expression Kit uses an optimized human cell extract, a reaction buffer containing the cellular components required for protein synthesis. When supplemented with the included accessory proteins, amino acid mix, RNA polymerase, and a DNA template cloned into the cloning vector, this system can synthesize protein for up to 6 hours. We recommend wearing gloves and using nuclease-free tubes and tips to avoid introducing nucleases to your samples.

Template preparation

For proper expression, all templates must contain the T7 promoter, an initiation codon, and an EMCV internal ribosome entry site (IRES) to facilitate high levels of *in vitro* protein expression. The final concentration of template plasmid should be 300 ng/μL. For a 20 μL protein synthesis reaction, using 0.3 μg template plasmid DNA is enough. Using a positive control vector to verify protein synthesis can be useful when unfamiliar with cell-free transcription-translation protocols.
The full length of the cloning vector is 3355 bp. Below shows the MCS region of the cloning vector.

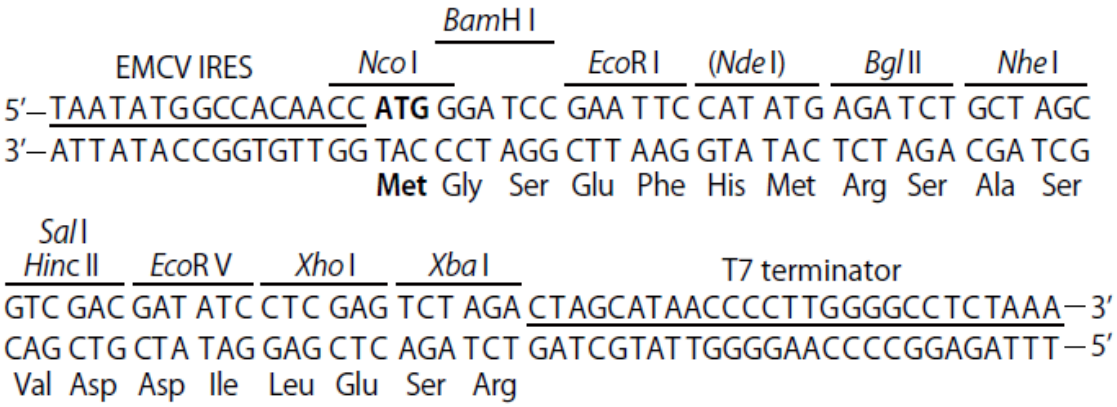


Fig 1 MCS region of the cloning vector. Design a primer containing the underlined 15-base sequence including the initiation codon (ATG), 5'-ATggCCACAACCATg-N-terminal coding sequence of the ORF-3'; design a primer containing the underlined 18-base sequence including the termination codon (TCA), 5'-gTTATgCTAgTCTAgTCA-C-terminal coding sequence of the ORF-3'.

Reactions

The standard volume is 20 µL per reaction. Thaw the Cell Lysate, Accessory Proteins, Reaction Buffer, Amino Acid Mix, and plasmid DNA on ice. Do not exceed five cycles of freezing and thawing.

1. Assemble the reaction in a new tube in the following order

Reagents (one 20 µL reaction)	
Cell Extract	9 µL
Reaction Buffer	6 µL
Accessory Proteins	1 µL
Incubate the above reagents at room temperature for 10 min	
Amino Acid Mix	2 µL
T7 RNA Polymerase	1 µL
DNA Template	1 µL (0.3 µg)
Total	20 µL

2. Incubate the tube at 32 °C for 1-6 hours. Generally, a reaction time of 3 hours is recommended, but reactions may be extended to up to 6 hours.

3. Use samples for analysis or purification or freeze at -20°C for use at a later time. The yield of the target protein will vary.

   NOTE: the yield of protein produced in cell-free systems is generally dependent on many factors, including:
   - Size of the protein
   - The sequence of the gene of interest
   - Expression of protein as a fusion with an N- or C-terminal tag
   - Optimized codon usage
   - Quality of the DNA template

Sample Images

Example 1

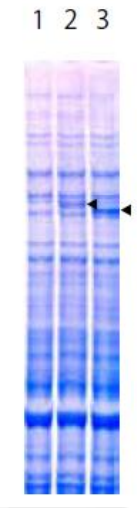


Fig 2 SDS-PAGE of large size proteins expressed using our kit with a standard protocol.

Lane 1, Negative Control. Lane 2, Human Dicer (200 kDa). Lane 3, Human eIF4G (170 kDa).

Example 2

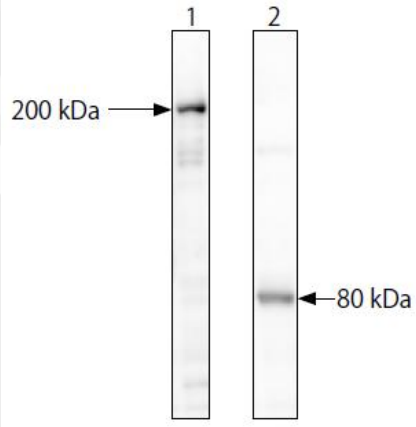
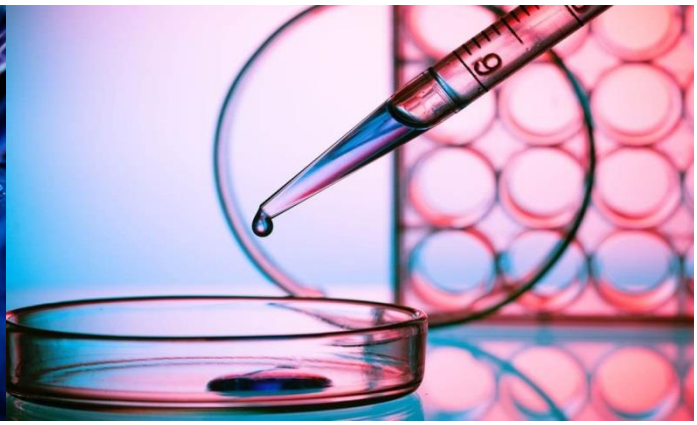


Fig 2 Western blot analysis of 200 kDa human dicer protein (lane 1) and 80 kDa human Ago2 protein (lane 2).

Find out more at **www.creative-biolabs.com**

Contact Us

For more information or technical assistance, please call, write, fax, or email to the following address:

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