Cell-Free Protein Synthesis Kit For VHH

REF: EG26310-S/M

Storage Condition

Store at -80℃ . Valid for 12 months.

Dry ice transportation. After opening, solution A and solution B should be stored at -80℃ .

To avoid repeated freeze-thaw cycles that reduce protein synthesis activity, solution A and solution B can be aliquoted in single-use portions, snap-frozen in liquid nitrogen, and then stored at -80℃ .

Components

Component	EG26310S	EG26310M
Solution A	300 μl	2×750 μl
Solution B	600 μl	3×1 ml
CFPS-Control Plasmid I	2 μg	2 μg
CFPS-Control Plasmid II	2 μg	2 μg

Description

Cell-Free Protein Synthesis Kit For VHH is optimized for the in vitro expression of nanobodies (VHH). Built on a genetically engineered Escherichia coli extract, it reconstitutes a coupled transcription-translation system in which T7 RNA polymerase drives high-level expression of the target gene. Recognizing that VHH molecules are small, disulfide-bond rich, and prone to aggregation, we have optimized the redox environment, chaperone components, and reaction buffer formulation of this kit.

It enables the production of soluble, correctly folded VHH proteins that retain antigen-binding activity within a few hours. The kit supports plasmid DNA or linear DNA templates, is compatible with high-throughput screening in microplates and scale-up preparation, and is widely applicable to rapid VHH expression verification, mutant screening, protein preparation, and functional studies.

Application

The product is restricted for scientific research and shall not be used for clinical diagnosis or treatment, nor for food or pharmaceuticals.

Protocol

1. Gene construction

CFPS-Control Plasmid I is the sfGFP expression positive control supplied with this kit, used to verify whether the kit reaction system functions properly.

CFPS-Control Plasmid II is the VHH positive-control expression plasmid supplied with this kit and can also be used as a universal cloning vector backbone. The plasmid carries a T7 promoter-driven VHH expression cassette. Users can replace the VHH coding sequence and the tag sequence by homologous recombination, according to their experimental needs, to construct an expression plasmid for the target protein. The functional elements of this plasmid (the DNA sequence is provided in the plasmid map, available for download on our website) are shown in Figure 1; each element is described below:

Translation enhancer: encodes the MSKIK amino-acid sequence, located at the translation initiation region. By enhancing ribosome recruitment and assembly of the translation initiation complex, it markedly increases the expression yield of the target protein.

GFP11: a fluorescence detection tag based on split-GFP technology. It is non-fluorescent on its own but reconstitutes a complete, fluorescent GFP when complemented with the GFP1-10 fragment (not included in this kit; to be purchased separately). It enables rapid quantitative detection of expression level without electrophoresis or Western blotting.

Strep II: an eight-amino-acid affinity purification tag (WSHPQFEK) that binds specifically to Strep-Tactin / Strep-Tactin XT resin. Compared with the His tag, it offers higher binding affinity and lower nonspecific background, and its mild elution conditions (competitive elution with biotin) preserve protein activity to the greatest extent. It is recommended as the preferred tag for VHH purification.

When constructing VHH expression vectors, it is recommended to optimize the VHH gene for E. coli codon usage to improve translation efficiency and protein expression levels. CFPS Control Plasmid II can be used as the plasmid backbone: this vector carries an N-terminal MSKIK translation-enhancer element (this sequence remains fused in the mature protein) combined with a C-terminal GFP11 tag, which significantly improves the expression stability of the VHH protein. Users may freely choose the tag type according to their experimental needs, such as 6×His, TwinStrep, or Fc tags, etc.

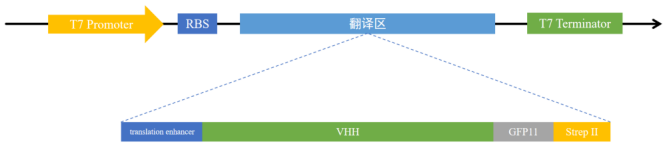


Figure 1 Functional elements of CFPS-Control Plasmid II

2. Template preparation

This kit supports three types of DNA templates for VHH expression: plasmid DNA, linear PCR products and RCA (rolling circle amplification) products. For initial expression or condition optimization, plasmid templates are recommended due to their high expression stability and good reproducibility. Linear PCR products require no cloning step, offering flexible and rapid operation, and are suitable for high-throughput screening. RCA products can rapidly generate large amounts of DNA template through isothermal amplification and are suitable for reaction systems requiring scale-up preparation. For detailed protocols and precautions for each template type, please refer to the Technical Manual.

3. Standard Protocol

Calculate the amount of each component based on the total reaction volume and the ratio of Solution A:Solution B = 1:2 (v/v). Add the reagents sequentially to the reaction vessel (e.g., a 2 ml centrifuge tube) under ice-bath conditions and mix gently by pipetting. Wear gloves and a mask throughout the operation, and use nuclease-free pipette tips and reaction vessels to avoid template degradation caused by nuclease contamination.

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Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com

Prepare the reaction system according to the table below. A 2 mL microcentrifuge tube, a 96-well microplate or a 96-well deep-well plate may be used as the reaction vessel, with a system volume of 50 μl, and the plate should be covered with a gas-permeable membrane. After gentle mixing, place in a constant-temperature shaker and carry out in vitro protein expression at 26 ℃ and 200 rpm. Usually, protein expression reaches its peak after 4~8 h of reaction, or an overnight reaction of 16 h can be selected.

Table 2 Reaction System

Reagent	Final Concentration	50 μl reaction system	100 μl reaction system
Solution A	30%	15 μl	30 μl
Solution B	60%	30 μl	60 μl
Template	5~10 μg/ml	5~10 μg/ml	5~10 μg/ml
Nuclease-Free water	\	Up to 50 μl	Up to 100 μl

4. Detection

After the reaction, approximately 1 μl of reaction liquid (for detecting total protein) or reaction supernatant (for detecting soluble protein) can be taken for SDS-PAGE electrophoresis to analyze the expression of the target protein. The VHH expression level with this kit is 0.1~0.5 mg/ml. When expression is low or gel staining is weak, the target band may be faint and easily misjudged as "no expression." In such cases, we recommend concentrating the sample by purification before electrophoresis.

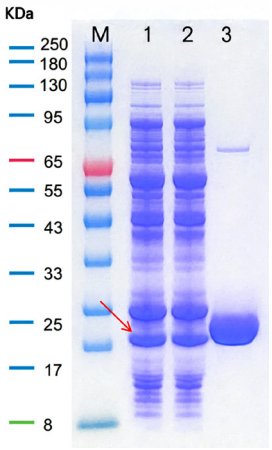


Figure legend:
Reference SDS-PAGE profile of the expression products of CFPS-Control Plasmid II in the cell-free protein synthesis system.
Lane 1: total protein;
Lane 2: soluble fraction;
Lane 3: purified sample.

Figure 2 Reference SDS-PAGE gel of the CFPS-Control Plasmid II expression product

If the expression vector carries a GFP11 tag, the more sensitive split-GFP technology can be used for rapid fluorescence detection: add 2 μl of GFP1-10 protein (not included in this kit) to 10 μl of cell-free reaction system and incubate at 26℃ for 4~6 h. After the reaction is complete, detect the fluorescence signal to evaluate the expression level; the GFP detection excitation wavelength is 485 nm and the emission wavelength is 528 nm.

5. Purification

Take 100 μl of CFPS reaction supernatant, add 50 μl of pre-equilibrated Strep-Tactin magnetic beads, and incubate with rotation at 4°C for 2 h. Place the tube on a magnetic stand and, once the beads are fully captured, discard the supernatant. Wash the beads 3-6 times with 100 μl of wash buffer, discarding the supernatant after each magnetic separation. Finally, add 50 μl of elution buffer, incubate at room temperature for 3-5 min, separate on the magnetic stand and collect the supernatant, which contains the purified VHH protein. For detailed instructions, refer to the user manual of the magnetic bead product.

6. Affinity Testing

After expression, the reaction supernatant (crude sample) can be directly used to perform preliminary screening of VHH molecular binding activity and affinity kinetics characterization by surface plasmon resonance (SPR) or bio-layer interferometry (BLI). A tag capture mode can be used to capture VHH onto the sensor surface, wash away nonspecific interfering components from the CFPS system, and then measure its interaction with the antigen to obtain kinetic parameters such as the association rate constant (ka), dissociation rate constant (kd), and equilibrium dissociation constant (KD).

Operating tips:

1. Prefer the reaction supernatant and avoid the reaction pellet to reduce the nonspecific binding background. Crude samples should be appropriately diluted before loading to reduce the refractive-index interference and nonspecific adsorption caused by the cell-free extract matrix.

2. Choose a capture sensor / chip matched to the tag (Strep-Tactin capture chip / sensor, or anti-His capture chip / sensor).

3. Crude samples are suitable for rapid primary screening and relative affinity ranking, and can effectively compare differences in binding activity among different VHH molecules. If higher-quality kinetic parameters suitable for formal quantitative analysis are required, we recommend performing the experiment with VHH protein samples purified via the Strep-II, His, or Fc tag.

4. For detailed instrument parameters, sensor hydration, and the capture-association-dissociation-regeneration workflow, please refer to the official operating manual of the corresponding SPR/BLI instrument.

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Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com