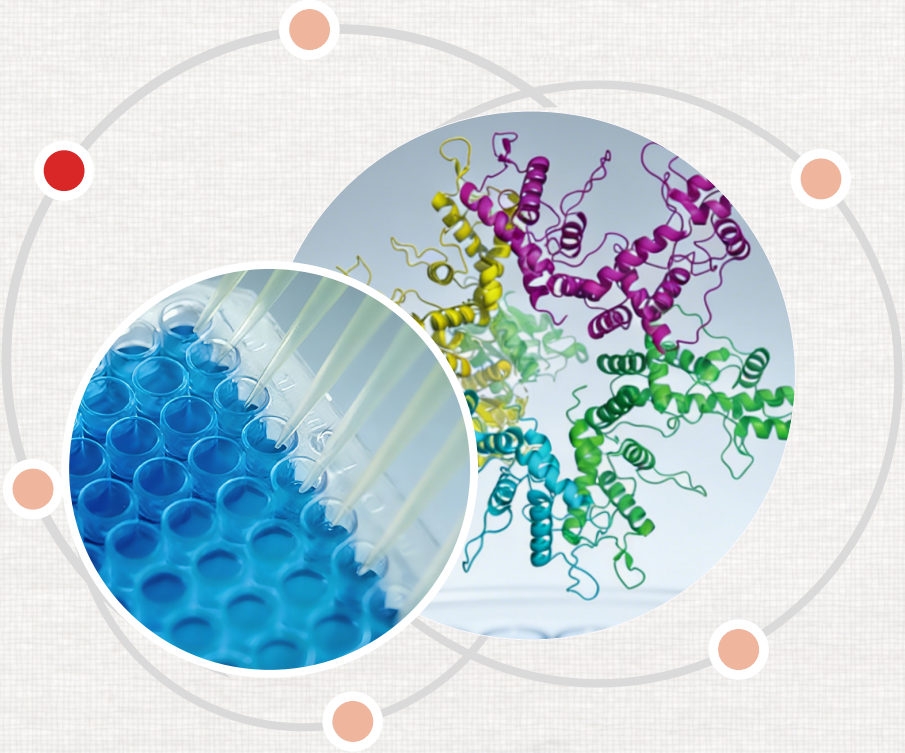
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BestEnzymes

Cell-Free Protein Synthesis (CFPS) Solution

Empowering AI-driven drug discovery (AIDD) and accelerating the design–validation cycle



ISO 13485 Quality management system certification

GMP

Why Choose Cell-Free Protein Synthesis Kit ?

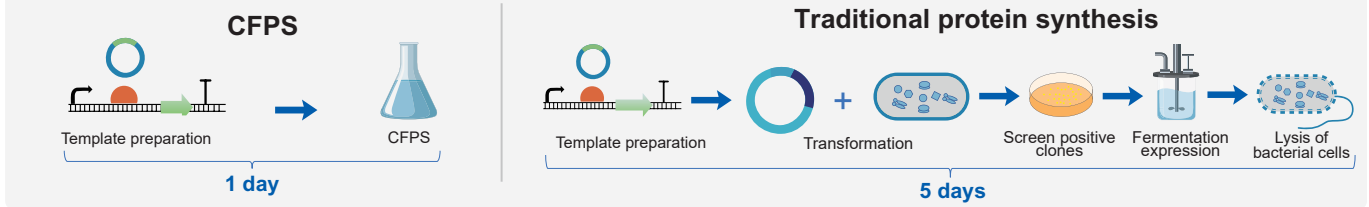
Breaking bottlenecks of traditional technology, improving the efficiency and flexibility of protein synthesis.

- Eliminates the inherent limitations of intracellular expression
- Enhances the controllability and flexibility of protein synthesis
- Shortens experimental cycles and improves high-throughput performance

Core Parameter Comparison: CFPS vs. Traditional Protein Synthesis

Comparison	CFPS	Traditional protein synthesis
Time	Express target proteins in 1-2 hours, with peak yield achieved in 8-16 hours.	Days or weeks.
Expression of special proteins	Express difficult-to-express proteins, such as cytotoxic proteins and disulfide-rich proteins.	Difficult to express cytotoxic, disulfide-rich and insoluble proteins
Convenience of operation	Simple and fast, just add DNA/RNA template.	Cell culture, transformation, induction, and lysis, etc.
High-throughput performance	Supports dual reaction systems (96-well plates / centrifuge tubes), enabling easy high-throughput screening while maintaining flexibility for various modifications.	Limited by the cell culture system, high-throughput expression and screening are difficult to achieve.

Process Comparison



Product Features

- **Rapid Expression**

  Target protein is obtained in 1–2 hours, with peak yield reached within 8–16 hours.

- **Specialized for Challenging Proteins**

  Enables efficient expression of cytotoxic, disulfide-rich (e.g., VHH) and insoluble proteins.

- **Flexible & High-Efficiency**

  From μL to L scale, enabling high-throughput screening.

- **Open System**

  Accommodates special requirements such as isotopic labeling and non-canonical amino acid incorporation.

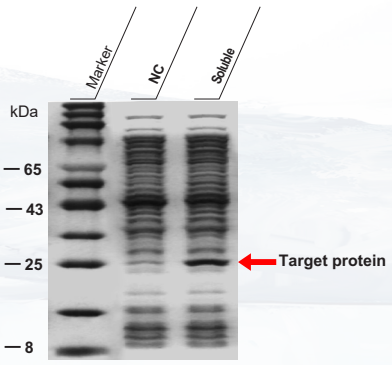
- **Broad Compatibility**

  Supports multiple templates including circular plasmids, linear DNA and mRNA, and is compatible with the T7 promoter system.

Performance Demonstration

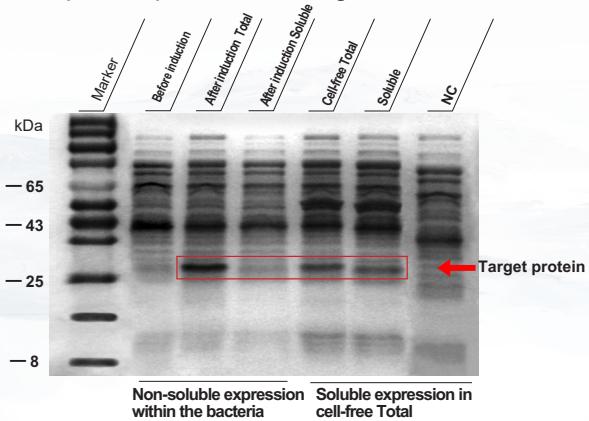
- **Cytotoxic protein**

  A restriction endonuclease



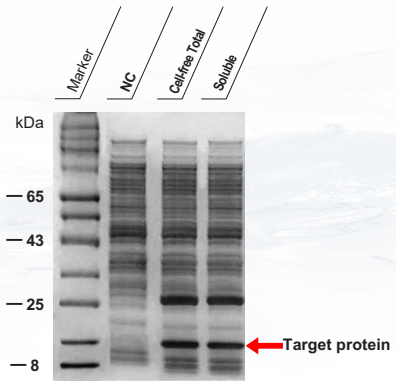
- **Misfolded or aggregated proteins**

  A protein prone to forming inclusion bodies



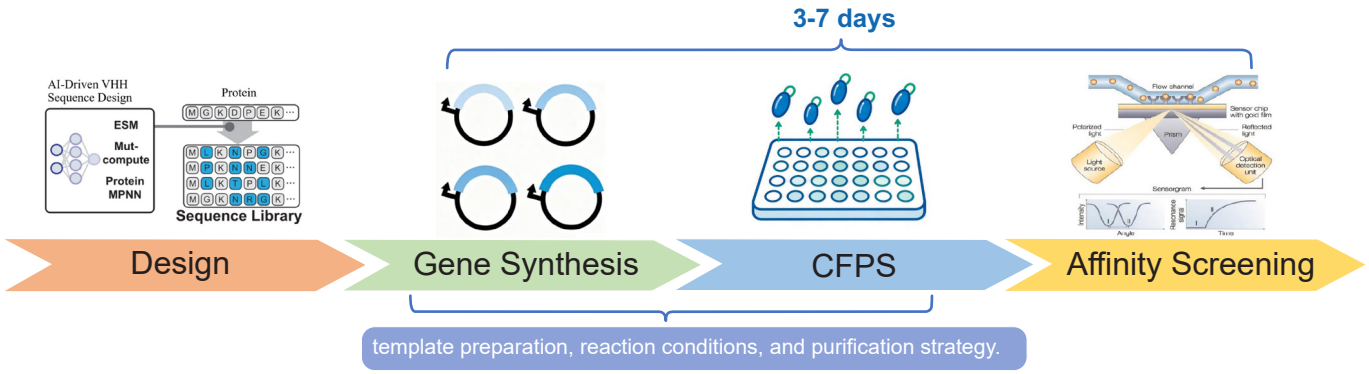
- **Disulfide-rich proteins**

  Luciferase



AI-Empowered Nanobody Discovery · High-Throughput VHH In Vitro Expression

BestEnzymes Biotech has developed standardized cell-free protein synthesis kits and solutions specifically for nanobodies (variable domain of heavy chain of heavy-chain antibody, VHH). No transformation, cell culture, or clone screening is required — sequences are directly converted into detectable proteins, greatly improving the efficiency of AI design validation.

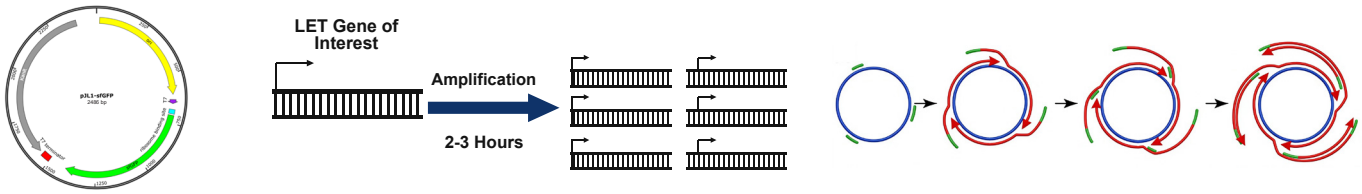


CFPS-based high-throughput VHH screening workflow

Performance Demonstration

Multiple Template Formats Can Be Directly Expressed

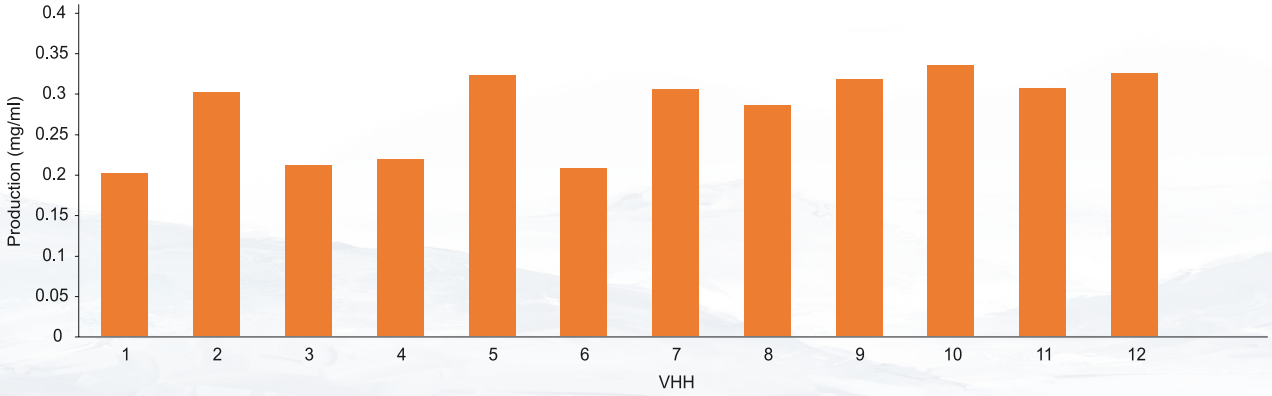
The CFPS system offers high template compatibility: circular plasmids, PCR linear products, and RCA (rolling circle amplification) products can all serve as expression templates without cloning and transformation steps. Even small amounts of template can rapidly produce sufficient protein, supporting high-throughput VHH in vitro screening and variant validation.



Plasmid PCR product RCA product

High Expression Success Rate

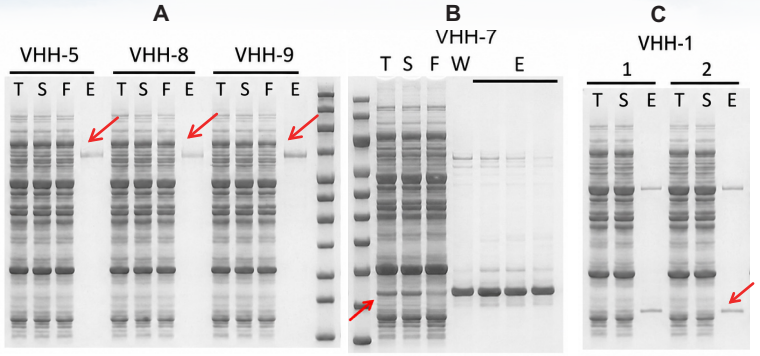
Twelve VHH variants reported in the literature were selected and expressed using PCR linear products as templates. In the BestEnzymes Biotech CFPS system, 12/12 were successfully expressed as soluble proteins, with half of the yields ≥ 0.3 mg/mL



Expression yield of multiple VHH variants in the CFPS system

Validation of Different Magnetic Bead Purification Routes

BestEnzymes Biotech validated three magnetic bead purification routes suitable for CFPS-VHH: His tag (Ni-NTA), VHH-Fc fusion (Protein A), and Strep-tag II. SDS-PAGE results demonstrate that all three routes effectively enrich the target protein, allowing flexible selection based on the desired construct format.



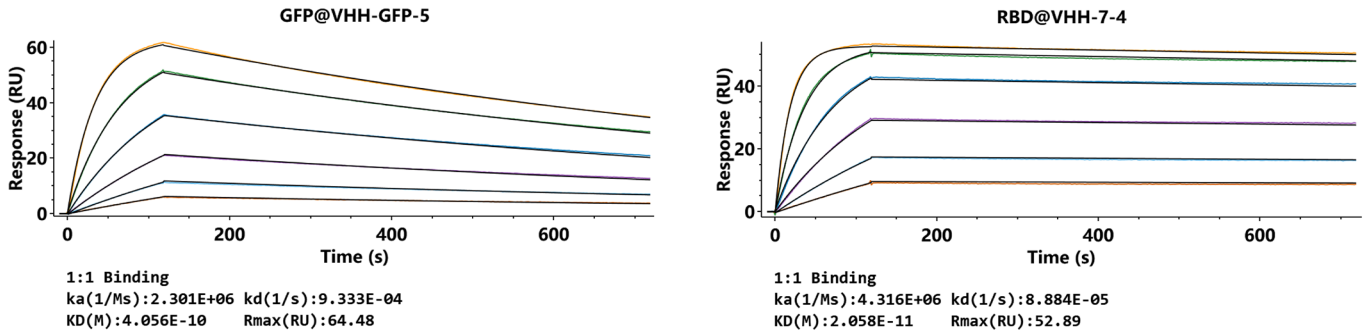
T: Cell-free Total S: Soluble F: Flow W: Wash E: Elute

A: VHH-Fc fusion protein purified with Protein A magnetic beads.

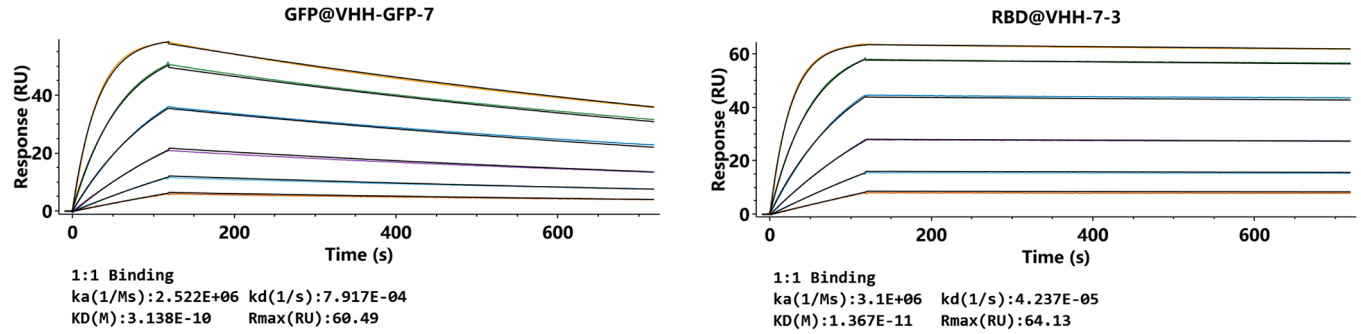
B: VHH-Fc fusion protein purified with Ni-NTA magnetic beads.

C: VHH-Fc fusion protein purified with Strep II magnetic beads.

Direct SPR Affinity Testing of CFPS-Produced VHH



Twin-Strep-tag VHH SPR binding—dissociation curve



Fc-tag VHH SPR binding—dissociation curve

Application

- Validation of AI-designed candidates
- Rapidly convert computationally screened sequences into detectable proteins in batches, obtaining experimental feedback required for the next round of design.
- VHH sequence and construct optimization
- Antibody discovery services
- Connect gene template preparation, cell-free expression, and affinity detection into a continuous workflow.
- Compare the expression and binding performance of different candidates, tags, and Fc fusion formats.

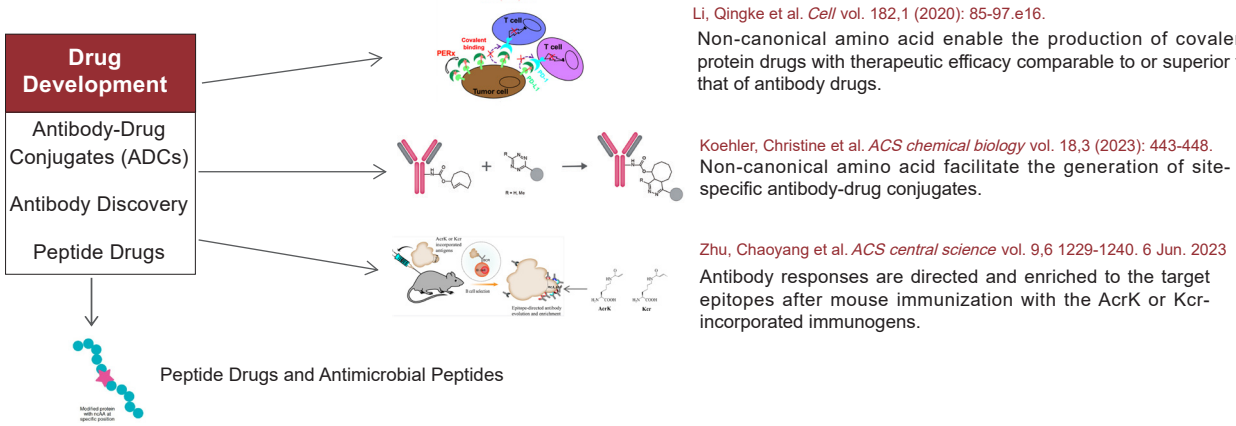
Expand Your Protein Universe: Incorporation of Non-Canonical Amino Acid

Non-Canonical Amino Acid, ncAA

In a broad sense, it refers to non-naturally occurring, synthetic amino acid. It generally denotes ncAA—those beyond the 20 standard amino acid—that are introduced into proteins via chemical synthesis or bioengineering methods.

Functional Applications of Non-Canonical Amino Acid

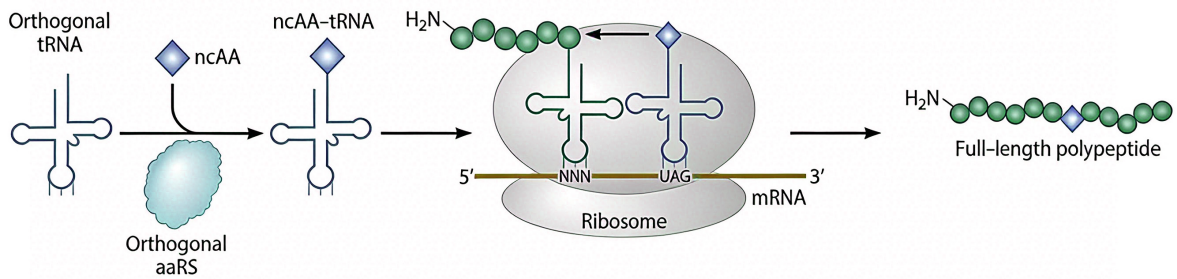
Introduce ncAAs into specific sites of target protein, endowing the protein with new biological characteristics including covalently binding with proximal proteins, carrying fluorescence, and mimicking specific protein post-translational modifications.



Examples of the Application of Non-Canonical Amino Acid in Drug Development

Incorporation Pathway of Non-Canonical Amino Acid

The codon at the intended insertion site for the ncAA within the coding gene is mutated to a termination codon (such as TAG). Subsequently, an orthogonal translation system for the ncAA is introduced into the cell-free protein synthesis system. This system comprises the $tRNA^{TAG}$, the specific ncAA, and the corresponding aminoacyl-tRNA synthetase (aaRS), thereby enabling the site-specific insertion of the ncAA during protein translation.



Schematic Diagram of ncAA Insertion Using an Orthogonal Translation System

Why is the Cell-Free Protein Synthesis System More Suitable for ncAA Incorporation?

- The cell-free environment avoids the degradation of ncAA by intracellular metabolism.
- Enables precise control over the concentration and time of ncAA.
- Free from cytotoxicity constraints, it enables the expression of proteins that are toxic to cells.
- Facilitates the optimization of reaction conditions to improve the incorporation efficiency of ncAA.



BestEnzymes Biotech has developed three cell-free protein synthesis kits for non-canonical amino acid (ncAA), enabling site-specific incorporation of p-acetyl-L-phenylalanine (pAcF), p-azido-L-phenylalanine (pAzF), or Nε-crotonyl-L-lysine (Kcr) into target proteins using the amber codon.

Product

Basic Versions

Standard lysate system covering different target proteins.

1.1	Standard	Most conventional proteins and cytotoxic proteins
1.2	Pro	Insoluble proteins
1.3	Max	Disulfide-bond-rich proteins and antibody fragments

Specialized Versions

Functional enhancements based on the basic system.

2.1 | ncAA protein Kit

pAzF/pAcF/Kcr/AcrK/NaeK

Applications: site-specific protein labeling, crosslinking, epigenetic research, drug development, etc.

2.2 | VHH version

VHH/VHH-Fc

Applications: high-throughput screening and rapid preparation of nanobodies.

Ordering Information

REF No.	Product Name	Specs
EG24110S/M/L	2× S705 HiFi Master Mix	1ml/5ml/20ml
EG25109S/M	phi29 II DNA Polymerase	500U/2500U
EG26310S/M	Cell-Free Protein Synthesis Kit For VHH	20 rxns/100 rxns
EG24301S/M	Cell-Free Protein Synthesis Kit	20 rxns/100 rxns
EG24302S/M	Cell-Free Protein Synthesis Kit (Lyophilized)	20 rxns/100 rxns
EG25301S/M	Cell-Free Protein Synthesis Kit Pro	20 rxns/100 rxns
EG25303S/M	Cell-Free Protein Synthesis Kit Pro (Lyophilized)	20 rxns/100 rxns
EG25302S/M	Cell-Free Protein Synthesis Kit Max	20 rxns/100 rxns
EG25304S/M	Cell-Free Protein Synthesis Kit Max (Lyophilized)	20 rxns/100 rxns
EG25330S	Cell-Free Protein Synthesis Kit (pAcF)	20 rxns
EG25331S	Cell-Free Protein Synthesis Kit (pAzF)	20 rxns
EG25332S	Cell-Free Protein Synthesis Kit (Kcr)	20 rxns

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