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BestEnzymes

Cell-Free Protein Synthesis Kit

—— Say goodbye to the long fermentation period!

- **Rapid Expression:** Target protein obtained in 1–2 hours, with peak yield reached within 8–16 hours.
- **Broad Compatibility:** Support multiple templates including circular plasmids, linear DNA and mRNA, compatible with the T7 promoter system.
- **Specialized for Challenging Proteins:** Enables efficient expression of cytotoxic, disulfide bond-rich and insoluble proteins.
- **Flexible & High-Efficiency:** Dual reaction systems (microplate / centrifuge tube) support high-throughput screening.
- **Open System:** Accommodates special requirements such as isotopic labeling and non-canonical amino acids incorporation.

2000+
Clean production plant (m²)

9000+
R&D and production base (m²)

13485
Quality management system certification

Why Choose Cell-Free Protein Synthesis Kit ?

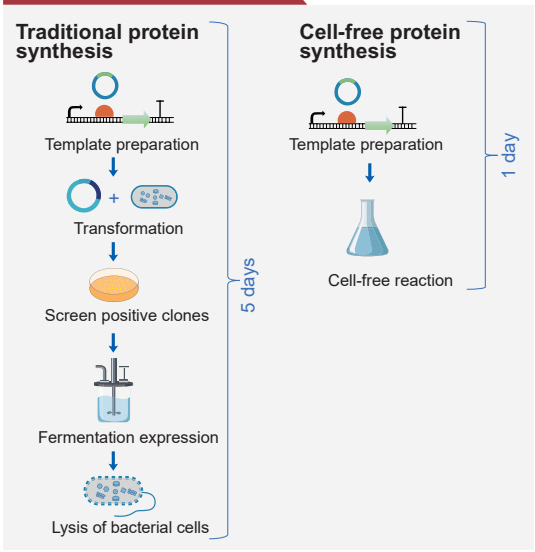
Breaking bottlenecks of traditional technology, improving the efficiency and flexibility of protein synthesis.

- 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: Cell-Free vs. Traditional Protein Synthesis

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

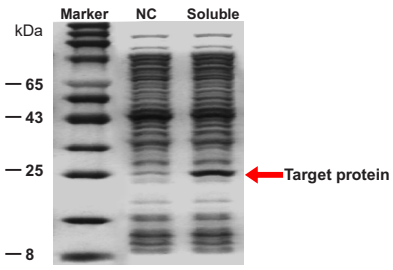
Process Comparison



Performance Demonstration

• Cytotoxic protein

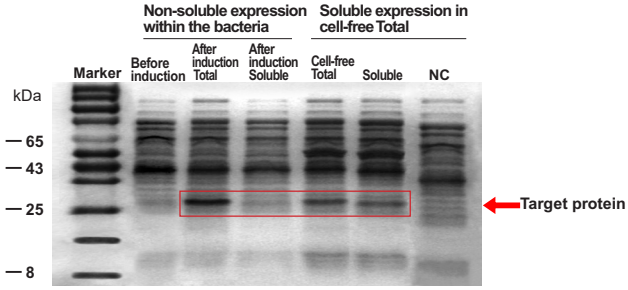
A restriction endonuclease



The soluble expression of a particular restriction endonuclease in the cell-free expression system.

• Misfolded or aggregated proteins

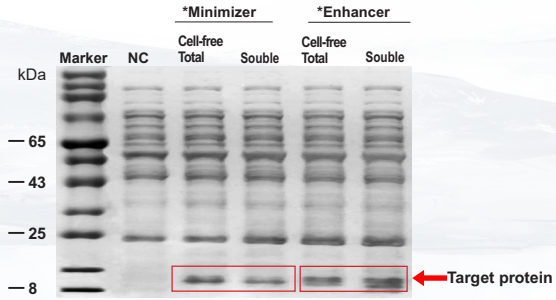
A protein prone to forming inclusion bodies



Proteins that tend to form inclusion bodies in *E. coli* cells are expressed solubly in the cell-free expression system.

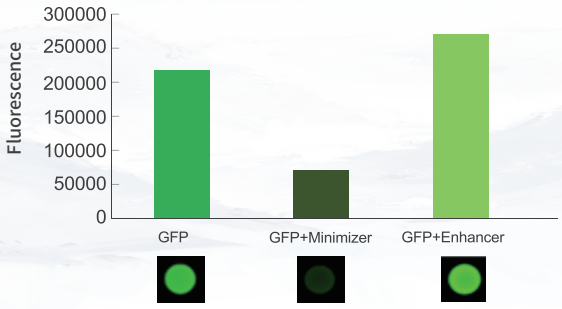
• Disulfide-rich proteins

GFP Nanobody



*Enhancer: GFP Fluorescence-enhanced Nanobody
*Minimizer: GFP Fluorescence-attenuated Nanobody

Functional assay



Two GFP-specific nanobodies were successfully expressed in a soluble form using a cell-free expression system. Upon binding to green fluorescent protein (GFP), both nanobodies retained their functional activity, demonstrating either fluorescence enhancement or suppression.

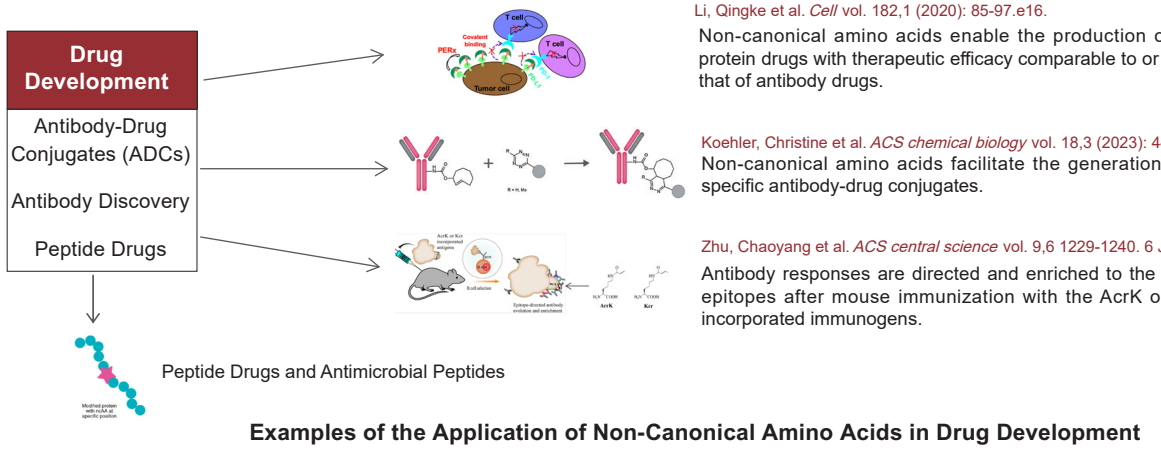
Expand Your Protein Universe: Incorporation of Non-Canonical Amino Acids

Non-Canonical Amino Acids, ncAAs

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

Functional Applications of Non-Canonical Amino Acids

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.



Li, Qingke et al. *Cell* vol. 182,1 (2020): 85-97.e16.
Non-canonical amino acids enable the production of covalent protein drugs with therapeutic efficacy comparable to or superior to that of antibody drugs.

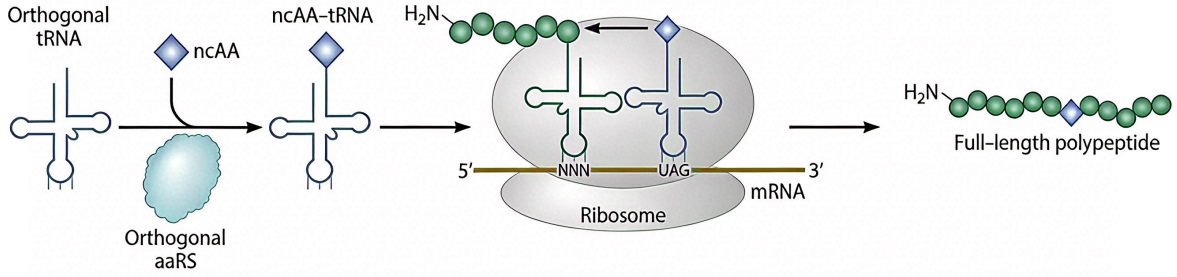
Koehler, Christine et al. *ACS chemical biology* vol. 18,3 (2023): 443-448.
Non-canonical amino acids facilitate the generation of site-specific antibody-drug conjugates.

Zhu, Chaoyang et al. *ACS central science* vol. 9,6 1229-1240. 6 Jun. 2023
Antibody responses are directed and enriched to the target epitopes after mouse immunization with the AcrK or Kcr-incorporated immunogens.

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

Incorporation Pathway of Non-Canonical Amino Acids

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



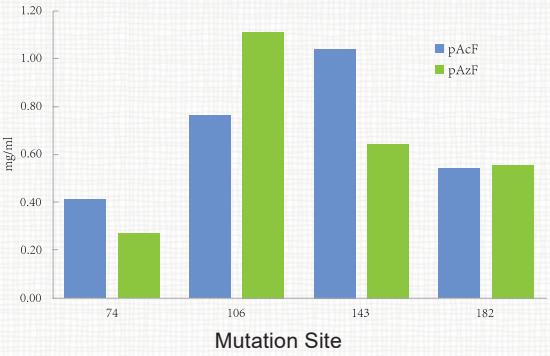
Schematic Diagram of ncAAs Insertion Using an Orthogonal Translation System

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

- The cell-free environment avoids the degradation of ncAAs by intracellular metabolism.
- Enables precise control over the concentration and time of ncAAs.
- 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 ncAAs.

Solution

BestEnzymes Biotech has launched a specialized cell-free protein synthesis kit compatible with p-acetyl-L-phenylalanine (pAcF),p-azido-L-phenylalanine (pAzF), and Nε-crotonyl-L-lysine (Kcr), enabling highly efficient incorporation of ncAAs and providing dedicated solutions for protein modification and engineering research.



BestEnzymes Biotech has developed three cell-free protein synthesis kits for non-canonical amino acids (ncAAs), 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 Selection Guide

Product name	Cell-Free Protein Synthesis Kit	Cell-Free Protein Synthesis Kit Pro	Cell-Free Protein Synthesis Kit Max
Plasmid / Linear template	√	√	√
Insoluble protein	/	√	√
Disulfide bond	/	/	√
Yield	Up to 3 mg/ml	Up to 2 mg/ml	Up to 2 mg/ml
Application	Suitable for standard protein expression. It enables high-efficiency production of proteins with diverse molecular weights (such as Green Fluorescent Protein, β-galactosidase, polymerases)	Suitable for expressing insoluble protein. Based on standard systems, we added chaperone proteins to enhance folding, improve solubility, and reduce the formation of inclusion bodies.	Suitable for expressing diverse proteins, particularly disulfide-rich proteins, including full-length antibodies, nanobodies, antibody fragments, cytokines, and membrane proteins.

Ordering Information

REF No.	Product Name	Specs
EG24302S	Cell-Free Protein Synthesis Kit (Lyophilized)	20 rxns
EG24302M	Cell-Free Protein Synthesis Kit (Lyophilized)	100 rxns
EG25303S	Cell-Free Protein Synthesis Kit Pro (Lyophilized)	20 rxns
EG25303M	Cell-Free Protein Synthesis Kit Pro (Lyophilized)	100 rxns
EG25304S	Cell-Free Protein Synthesis Kit Max (Lyophilized)	20 rxns
EG25304M	Cell-Free Protein Synthesis Kit Max (Lyophilized)	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

GMP ISO 13485

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