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BestEnzymes

PRODUCT FOR BIOPHARMA

—— Supporting Drug R&D and Manufacturing



ISO 13485 Quality management system certification

GMP

In every critical stage of developing and manufacturing innovative therapeutics—such as antibody drugs, nucleic acid drugs, and cell and gene therapy (CGT)—precise, efficient, and reliable molecular biology tools are indispensable.

At BestEnzymes Biotech, we are dedicated to creating advanced molecular biology tools to support the discovery of these technologies. Our extensive range of high-quality reagents are designed to meet the rigorous demands of the biotherapeutic industry.

Why Choose Us?

- **Strict Quality Control:** Full process quality management system, offering GMP-grade products and supporting DMF filing.
- **Comprehensive Product Range:** Nearly 200 types of full-range enzyme preparations, covering DNA/RNA/protein operations. One-stop product portfolio from R&D to production.
- **Customization Support:** Providing customization for large packaging and GMP-grade products.

PRODUCT FOR BIOPHARMA ——Product Selection Guide

RNA Therapeutics

❶ Template Generation

- Restriction Endonucleases
- T4 DNA Ligase
- Ultra T4 DNA Ligase
- M-MLV Reverse Transcriptase
- High Fidelity Polymerase PCR Mix
- Golden Gate Assembly Kit
- Gibson DNA Assembly Mix
- phi29 II DNA Polymerase
- TelN Protelomerase
- BsaI, GMP Grade
- BspQI, GMP Grade
- XbaI, GMP Grade
- XhoI, GMP Grade
- NheI, GMP Grade

❷ *In Vitro* Transcription (IVT)

- T7 High Yield RNA Synthesis Kit
- T7 RNA Polymerase, GMP Grade
- High T7 RNA Polymerase
- Murine RNase Inhibitor, GMP Grade
- DNase I, RNase-free
- DNase I-ST, GMP Grade
- Pyrophosphatase, Inorganic (Yeast, GMP Grade)
- Thermostable Inorganic Pyrophosphatase
- NTP, Modified NTP
- Cap1 analogue AG
- Poly(A) RNA Polymerase
- Proteinase K

❸ Small RNA

- dsRNA Ligase
- T4 Polynucleotide Kinase

❹ Circular RNA

- T4 RNA Ligase 1

❺ Analysis & Quality Inspection

- RNase GG
- RNase T1
- Thermostable RNase T1
- RNase H
- Thermostable RNase H
- MazF
- RNase A
- BsaI ELISA Kit
- XhoI ELISA Kit
- XbaI ELISA Kit
- BsmBI ELISA Kit

❻ Auxiliary Reagents

- Ready To Use Instant Granules
- Nucleic Acid Stain
- RNA Marker
- DNA Marker

Antibody Drugs

❶ Molecular Cloning Construction

- Restriction Endonucleases
- High Fidelity Polymerase PCR Mix
- T4 DNA Ligase
- Golden Gate Assembly Kit
- Gibson DNA Assembly Mix
- phi29 II DNA Polymerase

❷ AIDD

- **Cell-Free Protein Synthesis Kit**

❸ protein purification

- DNase I, RNase-free
- DNase I-ST, GMP Grade
- RNase A
- Omni-nuclease (Benzonase)
- TEV Protease (with His-tag)
- SUMO Protease

❹ Quality Control

- Benzonase ELISA Kit
- PNGase F

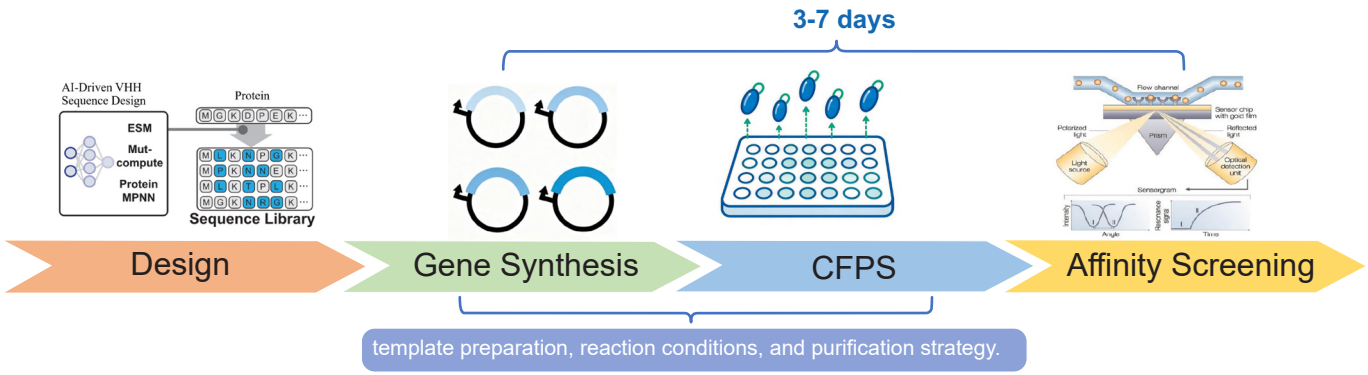
AIDD Antibody Screening & Development

Cell-Free Protein Synthesis Kit is an extract-based transcription/translation system derived from E. coli cells, which enables rapid, one-pot conversion of DNA/RNA templates to functional proteins in vitro. The kit employs a T7 promoter-based system, supporting multiple templates including circular plasmid DNA, linear DNA, and mRNA. It is particularly suitable for:

- Efficient production of difficult-to-express proteins (such as cytotoxic proteins).
- Correct folding of complex structured proteins which contain disulfide-rich proteins.
- Special modifications such as isotope labeling and incorporation of non-canonical amino acids.

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.

In addition, proprietary kits specifically designed for the insertion of three non-canonical amino acids—pAzF, pAcF and Kcr—are available for researchers.



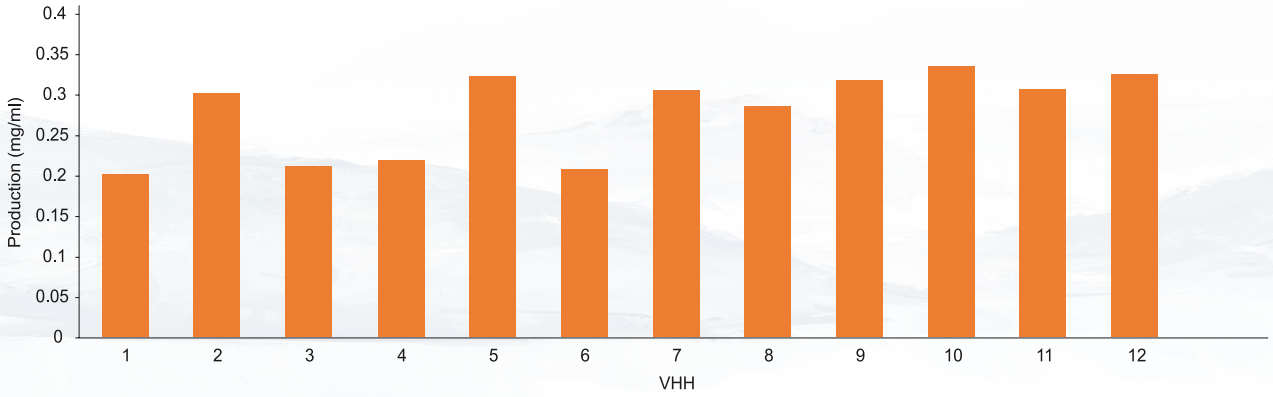
CFPS-based high-throughput VHH screening workflow

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.

High Expression Success Rate

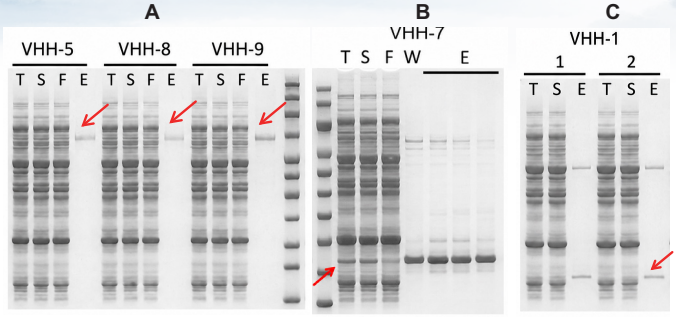
Twelve VHH variants reported in the literature were selected and expressed using PCR linear products as templates. In the BestEnzymes Biotech CFPS system, 12/12 were successfully expressed as soluble proteins, with half of the yields ≥ 0.3 mg/mL



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.



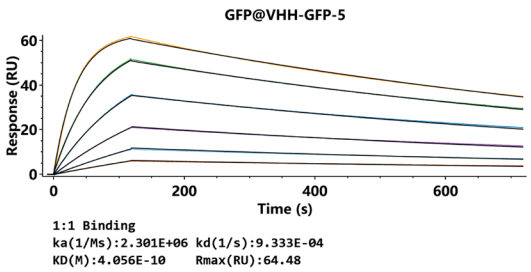
T: Cell-free Total S: Soluble F: Flow W: Wash E: Elute

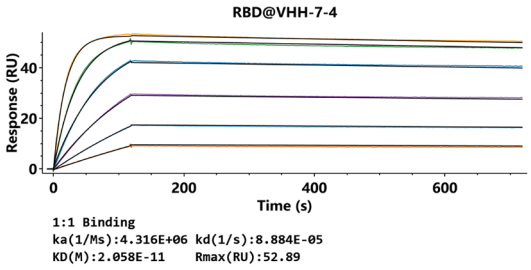
A: VHH-Fc fusion protein purified with Protein A magnetic beads.

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

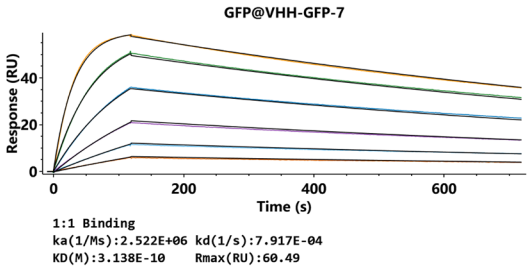
C: VHH-Fc fusion protein purified with Strep II magnetic beads.

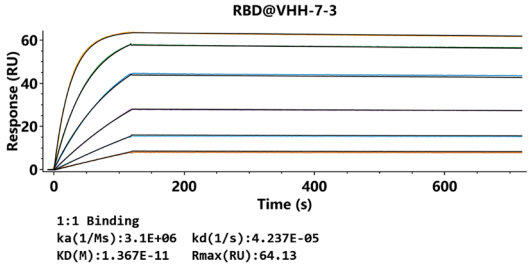
Direct SPR Affinity Testing of CFPS-Produced VHH





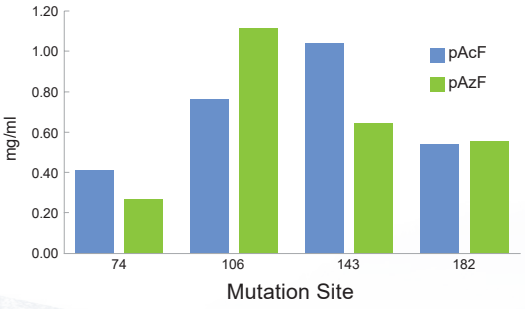
Twin-Strep-tag VHH SPR binding—dissociation curve





Fc-tag VHH SPR binding—dissociation curve

CFPS for Non-Canonical Amino Acid Incorporation



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

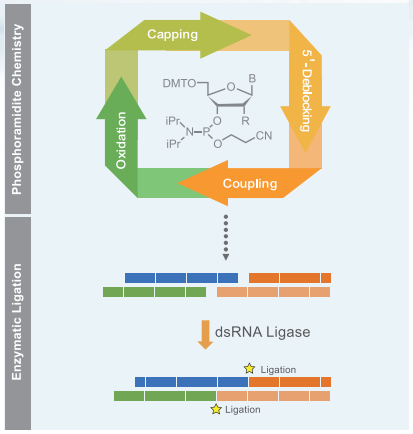
Applications: site-specific protein labeling, crosslinking, epigenetic research, drug development, etc.

2.2	**VHH version**	VHH/VHH-Fc

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

Enzymatic Synthesis Solution for Small RNA

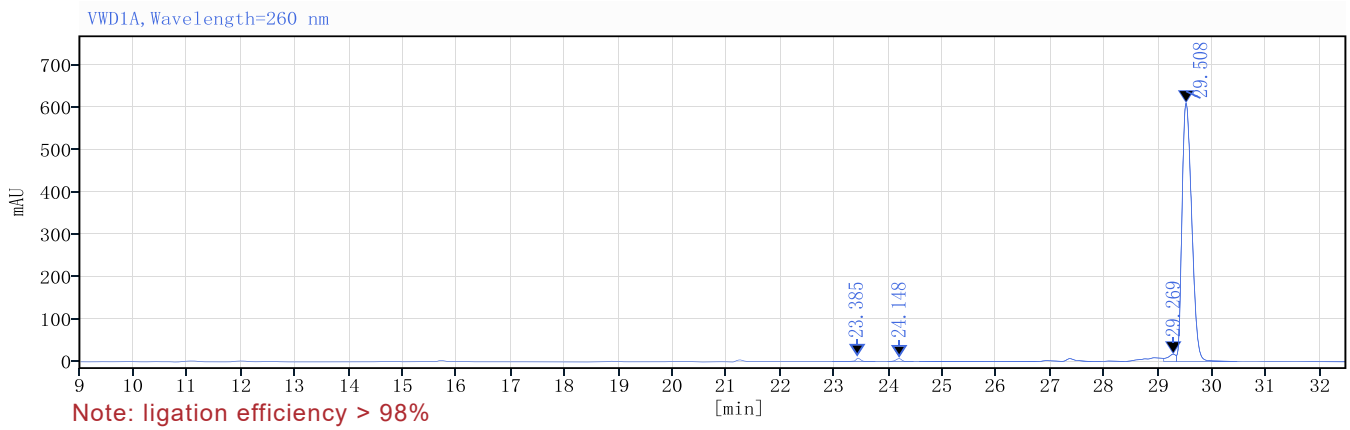
siRNA Manufacturing: Chemical Synthesis vs. Enzymatic Ligation—Process & Cost Comparison



	Solid-Phase Oligonucleotide Synthesis (SPOS)	Enzymatic Ligation
Principle	Nucleotides are added sequentially on a solid support; long chains are built via chemical bond formation	Chemical synthesis of short fragments + enzyme-catalyzed ligation; modular assembly
Core Steps	Single-strand synthesis → Annealing → Purification	Fragment synthesis → Annealing → Enzymatic ligation → Purification
Theoretical Yield	Decreases exponentially with chain length	High (modular ligation avoids cumulative loss)
Major Impurities	N−x / N+x fragments, starting materials, process degradation products, etc.	Unligated fragments, enzyme protein, starting materials, process degradation products, etc.
Cost	High	Higher at small scale; Lower at large scale
Eco-friendliness	Poor	Better
Applications	Short -chain RNA (~20-mer)	Long-chain RNA (>30-mer) and complex structures

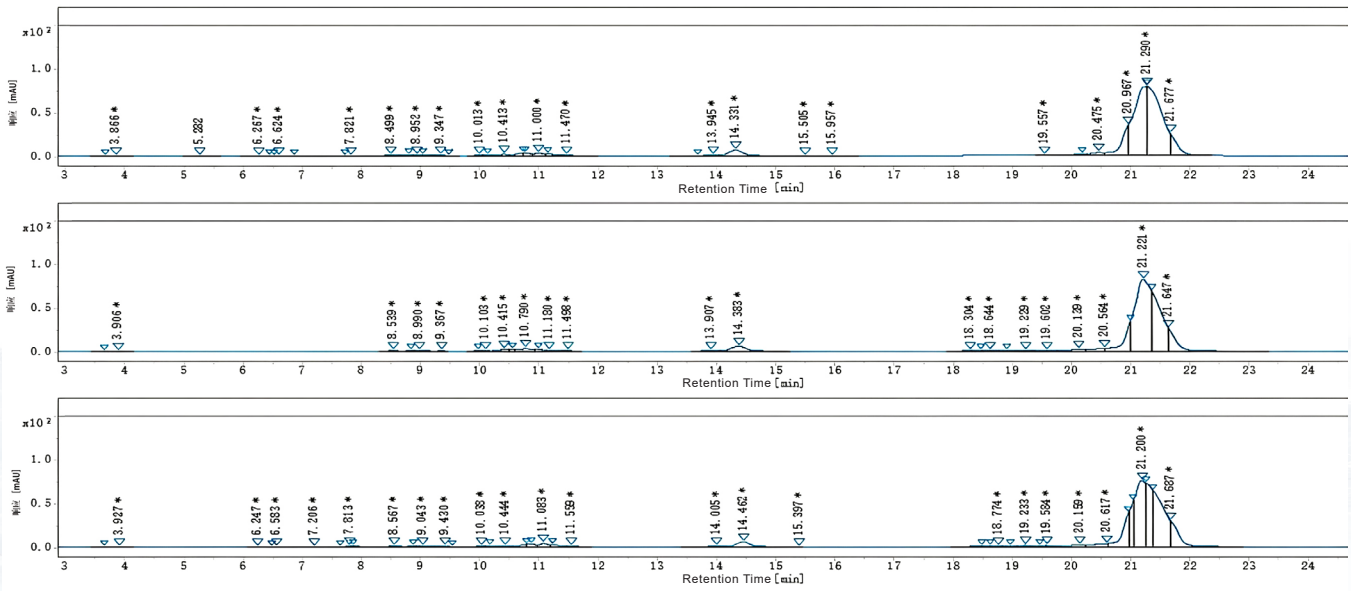
Leveraging an AI-driven enzyme engineering platform, we have engineered dsRNA ligase in a targeted manner and built a highly diverse mutant enzyme library, with patent applications filed. Together with complementary reaction processes, we offer a complete enzymatic ligation solution.

dsRNA Ligase Case Studies



Note: ligation efficiency > 98%

Single-target siRNA enzymatic ligation (Inclisiran)



Note: ligation efficiency > 95%

Dual-target siRNA enzymatic ligation

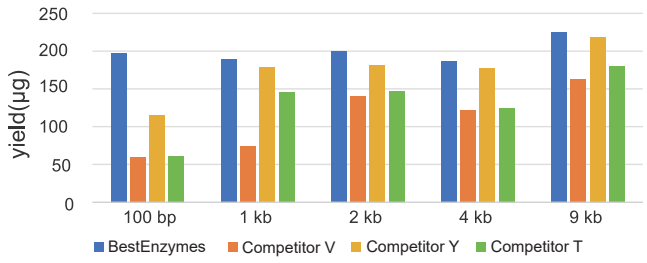
mRNA *In Vitro* Transcription (IVT)

Example of Quality Standards for GMP-grade Raw Material Enzymes

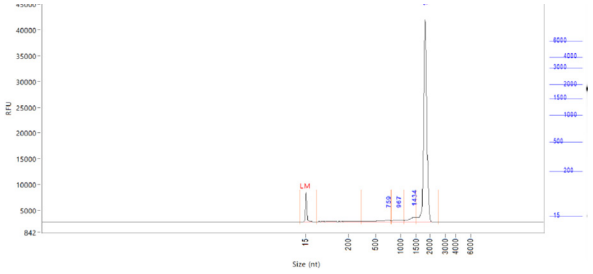
Quality Control	Quality Standard	Detection Method
Protein Purity	≥ 95%	SDS-PAGE
Non-specific Endonuclease Activity	Supercoiled plasmid conformational change < 20%	20 U enzyme was incubated with supercoiled plasmid at 37℃ for 4 hours
DNase Activity	No dsDNA degradation observed	20 U enzyme was incubated with dsDNA fragments at 37℃ for 16 hours
RNase Activity	RNA degradation ≤ 10%	20 U enzyme was incubated with RNA at 37℃ for 1 hour
Host Cell DNA	< 10 copies/20 U	ChP(2025) Volume IV, Detection of Exogenous DNA Residues, Method 3 (General Chapter 3407)
Host Cell Protein	< 50 ppm	ChP(2025) Volume IV, Measurement of E. coli Host Cell Proteins (General Chapter 3412)
Bacterial Endotoxin	< 10 EU/mg	ChP(2025) Volume IV, Bacterial Endotoxins Test, Method 1 (General Chapter 1143)
Mycoplasma	Negative	Mycoplasma test kit
Heavy Metals	< 10 ppm	ChP(2025) Volume IV, Limit Test for Heavy Metals, Method I (General Chapter 0821)
Microbial Limit	The total aerobic microbial count is below 5 cfu/ml, and the total combined yeasts/molds count is below 5 cfu/ml.	ChP(2025) Volume IV, Microbial Limit Tests for Non-Sterile Products: Microbial Enumeration Method (General Chapter 1105)

Note: The above information is given as examples of Bsal, GMP Grade .

The T7 High Yield RNA Synthesis Kit is capable of synthesizing long or short RNA transcripts in large quantities from DNA templates containing a T7 promoter. The kit's T7 Enzyme Mix is optimized with RNase inhibitor and inorganic pyrophosphatase, ensuring highly efficient and robust RNA synthesis.The synthesized RNA is applicable for a wide range of downstream applications, including *in vitro* translation, RNA structure and function studies, RNase protection assays, probe hybridization, RNA interference, and more.



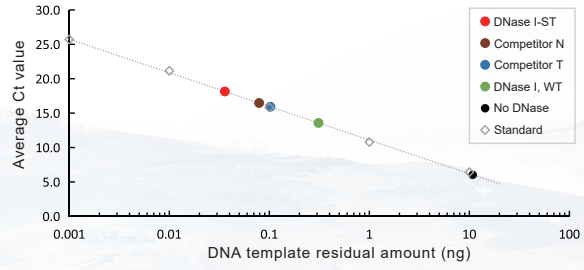
The relationship between different template lengths and the products yields.



As tested by customers，when using cap analogues for co-transcription, the integrity of the capped mRNA exceeds 85%.

DNase I-ST, GMP Grade——Through protein design and modification, BestEnzymes Biotech has developed a new salt-tolerant DNase I based on the wild-type DNase I. This product features:

- Significantly enhanced DNA affinity, enabling higher digestion efficiency for low-concentration DNA.
- High salt tolerance, which can efficiently degrade DNA even in solutions containing 300 mM salt.
- Recombinant expression, with no RNase contamination.
- Especially suitable for removing DNA templates after IVT reactions.



	Average Ct value	Residual DNA (ng)	Residual DNA ratio	DNA removal fold
DNase I-ST	18.1	0.036	0.332%	~300
Competitor N	16.5	0.079	0.732%	~130
Competitor T	15.9	0.102	0.948%	~100
DNase I, WT	13.6	0.311	2.875%	~30
No DNase	6.0	10.805	100%	-

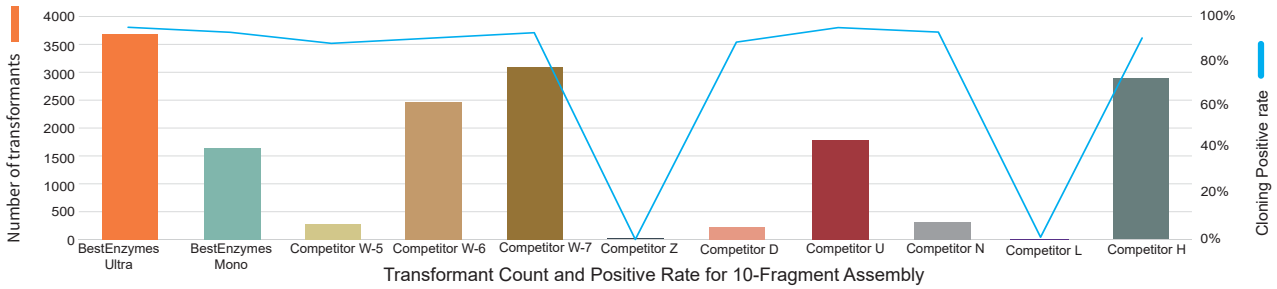
As shown in the image, *in vitro* transcription (IVT) reactions using different competitor systems—all with high salt concentrations—were performed using linearized plasmid DNA as template. After transcription, 2 U of DNase I were added. The RNA products were purified and recovered, and residual DNA was quantified by qPCR. The results demonstrate that BestEnzymes Biotech DNase I-ST achieved significantly higher DNA removal efficiency than the wild-type DNase I and competing products.

Template Generation

Restriction Endonucleases , GMP-grade Restriction Endonucleases (BsaI,BspQI,NheI,XhoI,XbaI). BestEnzymes offers multiple GMP-grade Endonucleases . We ensure full traceability of the production process and raw materials, with no antibiotics or animal-derived raw materials/excipients used. Strict control is implemented over process-related impurities (e.g., host cell protein, host cell DNA, non-specific endonuclease activity, DNase activity, RNase activity) as well as microbial limit and bacterial endotoxin, thus meeting the strict requirements for raw materials/excipients in the vaccine and drug production fields.

Meanwhile, BestEnzymes Biotech provides customization of GMP-grade restriction Endonucleases to meet personalized needs for plasmid templates.

Gibson DNA Assembly Mix and **Golden Gate Assembly Kit** are available for your selection. Support both single-fragment and multi-fragment assembly, with simple and convenient operation and high positive clone rate.



Phi29 II DNA Polymerase & TelN Protelomerase ——As core enzymes for Doggybone DNA™, they offer several advantages for mRNA template preparation: fast speed, high safety, flexible scale, good stability, and wide applicability.

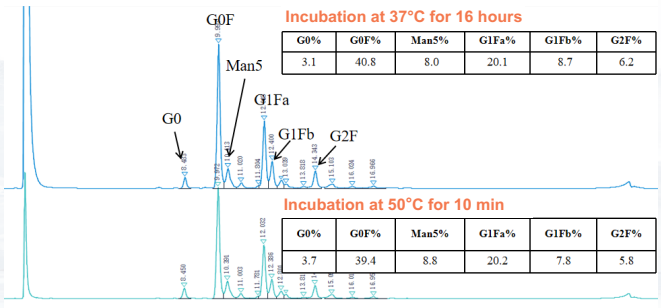
RNA Analysis

When performing *in vitro* transcription with both capping and tailing to generate complete mRNA, it's crucial to analyze the quality of the RNA product. To address this need, BestEnzymes offers a diverse array of RNase. These enzymes provide versatile options for RNA secondary structure analysis, capping rate evaluation, and comprehensive integrity assessment.

Product Name	Reaction Substrate	Recognition Sequence	Application
RNase A	Primarily single-stranded RNA	C/U	• Preparation of plasmids and genomic DNA • In combination with RNase T1
RNase T1/ Thermostable RNase T1	Single-stranded RNA	G	• Analysis of RNA secondary structure • mRNA quality analysis
RNase H/ Thermostable RNase H	The RNA chain in the RNA-DNA hybrid molecule	Positional preference	• Analysis of mRNA cap addition efficiency • Remove mRNA before synthesizing the second strand of cDNA
MazF	Single-stranded RNA	Unmodified ACA	• mRNA analysis
RNase GG	Single-stranded or multi-stranded RNA	GG	• mRNA analysis

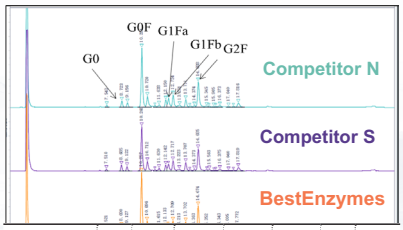
Antibody Quality Control

PNGase F is the most effective enzymatic method for removing almost all N-linked oligosaccharides from glycoproteins. PNGase F from BestEnzymes Biotech, recombinantly expressed in Escherichia coli, offers high purity and free from proteases or other glycosidases. It can be used either at 37 ° C using a standard reaction or at 50 ° C for a quick 10-minute reaction. In addition, A glycerol-free version of PNGase F (EG23304) is also offered for HPLC methods. PNGase F products are equipped with a His tag, facilitating their removal post-deglycosylation reaction.



G0%	G0F%	Man5%	G1Fa%	G1Fb%	G2F%
3.1	40.8	8.0	20.1	8.7	6.2
3.7	39.4	8.8	20.2	7.8	5.8

Glycosylation analysis of a monoclonal antibody treated under two reaction conditions showed no significant differences in glycan types or relative abundance.



Name	G0%	G0F%	Man5%	G1Fa%	G1Fb%	G2%	G2F%
Competitor N	3.25	30.23	7.36	5.5	7.33	4.52	16.59
Competitor S	3.49	27.16	7.4	5.07	7.13	5.68	16.82
BestEnzymes	3.71	27.89	7.92	5.14	7.21	6.54	16.34

Incubation at 37° C for 16 hours, glycosylation analysis of the bispecific antibody showed no significant differences in glycan types or relative abundance compared to competing products.

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BestEnzymes

BestEnzymes Biotech Co., Ltd

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

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