百 时 美
BestEnzymes

# mRNA *In vitro* Synthesis Solutions

Template Generation • Template Linearization
*In vitro* Transcription • RNA Analysis

# mRNA *In vitro* Synthesis Solutions

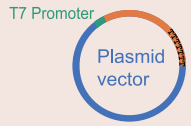
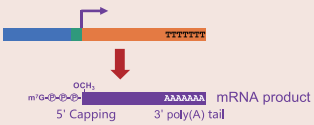
## Products manufactured in compliance with GMP regulations can meet diverse customer requirements.

### GMP Raw Material Quality Standards

| Quality Control | Quality Standard | Detection Method |
|---|---|---|
| Appearance | Colorless clear liquid | Visual Check |
| 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 CFU/mL |
| 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 BsaI, GMP Grade .

### mRNA Vaccine Manufacturing Process

| Stage | Schematic Diagram |
|---|---|
| 1. Acquisition of antigen genes |   |
| 2. Construction and amplification of Recombinant plasmid |   |
| 3. Linearization of circular plasmid template |  |
| 4. *In vitro* transcription (co-transcription with cap analogue ) |   |
| 5. Lipid nanoparticle encapsulation |  |

BestEnzymes Biotech offer a wide range of GMP and non-GMP grade raw materials, assisting in the research and production of mRNA vaccines.

### mRNA Synthesis Workflow Example & Available Products

| 1 Template Generation | 2 Template Linearization | 3 *In vitro* Transcription | 4 RNA Capping*and Tailing | 5 RNA Analysis |
|---|---|---|---|---|
| Restriction Endonucleases | BsaI, GMP Grade (GMP) | T7 High Yield RNA Synthesis Kit | Cap1 analogue AG | RNase A |
| 2× S705 HiFi Master Mix | XbaI, GMP Grade (GMP) | T7 RNA Polymerase (GMP) | Poly(A) RNA Polymerase | RNase T1 |
| Ultra / T4 DNA Ligase | XhoI, GMP Grade (GMP) | High T7 RNA Polymerase | | Thermostable RNase T1 |
| DNA Assembly Mix Ultra/ Mono | NheI, GMP Grade (GMP) | RNase Inhibitor, Murine (GMP) | | RNase H |
| Golden Gate Assembly Kit (BsmBI) | BspQI, GMP Grade (GMP) | DNase I，RNase-free | | Thermostable RNase H |
| Golden Gate Assembly Kit (BpiI) | Esp3I(BsmBI), ADCF | DNase I-ST, RNase-free (GMP) | | MazF |
| 2× Taq PCR Aurora Mix | EcoRI, ADCF | Pyrophosphatase, Inorganic (Yeast) (GMP) | | RNase GG |
| phi29 II DNA polymerase | BsaI ELISA Kit | Pseudo-UTP | | |
| TelN Protelomerase | XhoI ELISA Kit | $N^1$-Methyl-Pseudo-UTP | | |
| dNTPS | XbaI ELISA Kit | NTPs | | |
| | BsmBI ELISA Kit | | | |

*Accurately controlling poly(A) tail length using enzymatic methods is challenging, which is why the poly(A) sequence is typically incorporated directly into the plasmid template.

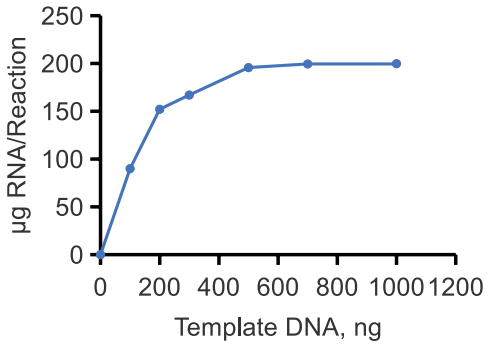
# *In vitro* Transcription

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.

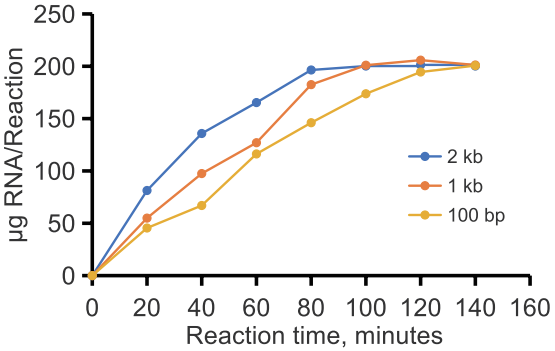
## Product Feature

- High yield,1 μg of linearized double-stranded DNA template can yield at least 150 μg of RNA.
- Fast rate，80 minutes will be sufficient to obtain the required amount of the product.
- Wide adaptability to template lengths (100 bp to 9 kb), suitable for preparing mRNA, CRISPR-required sgRNA, and other applications.
- Optimized reaction system, compatible with modified NTPs and cap analogs.

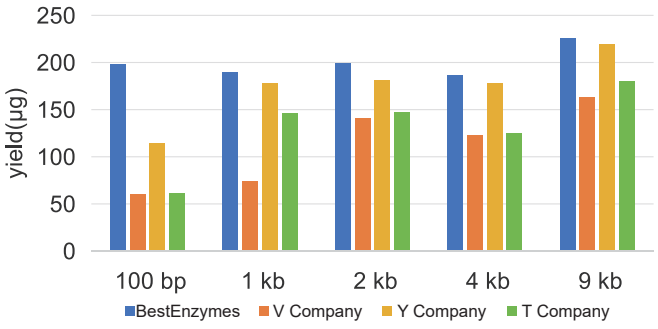
## Performance Demonstration



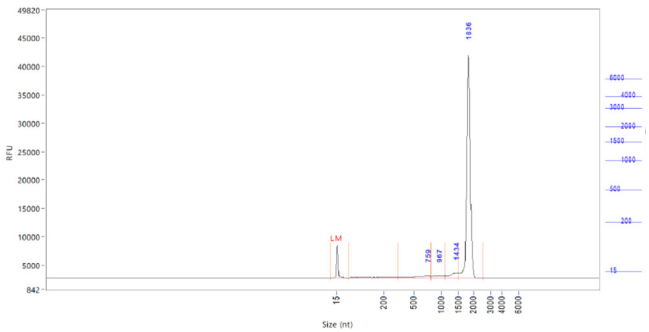

The relationship between amount of template and product.




The relationship between reaction time of different templates length and product output.




The relationship between different template lengths and the products output.



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

# Template Generation

When amplifying the target fragment, it is essential to select a high-fidelity DNA polymerase to ensure the accuracy of the template. BestEnzymes Biotech has launched the 2×S705 HiFi Master Mix, a high-fidelity PCR premix containing S705 High-Fidelity DNA Polymerase. This polymerase offers 70-fold higher fidelity than Taq polymerase and 5-fold faster amplification than Pfu polymerase, meeting the requirements for accurate template amplification.

BestEnzymes Biotech offers a comprehensive catalog including over a hundred restriction endonucleases, thermostable reverse transcriptase, T4 DNA Ligase (Fast), and Gibson assembly cloning kits, effectively addressing the needs for plasmid template construction.

# Template Linearization

When utilizing plasmid DNA as an *in vitro* transcription template, it's crucial to select appropriate restriction endonucleases to ensure complete digestion and linearization of the circular plasmid. BestEnzymes Biotech offers a diverse range of GMP-grade enzymes. We guarantee full traceability throughout our entire production process, including all raw materials and excipients. Our enzymes are completely free of antibiotics and any animal-derived components. Furthermore, we rigorously control process-related impurities such as host proteins, exogenous DNA, non-specific endonucleases, DNase, and RNase, as well as microbial limits and bacterial endotoxins. This stringent quality control meets the demanding raw material and excipient requirements for critical applications like vaccine and drug production and cell therapy.

Meanwhile, BestEnzymes Biotech provides GMP-grade restriction endonucleases customization to meet users' personalized needs for plasmid templates.

To address the issue of restriction endonuclease residue after plasmid template linearization, BestEnzymes Biotech pioneered the industry's first series of detection kits for restriction endonuclease residues. Currently, ELISA kits for individual enzymes including BsaI, XhoI, XbaI, and BsmBI are available, with new products to be gradually launched according to customer demands.

# 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 Biotech 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<br>Remove RNA from recombinant protein preparations RNA<br>In combination with RNase T1<br>Identify single-base mutations in DNA or RNA |
| **RNase T1/ Thermostable RNase T1** | Single-stranded RNA | G | RNA sequencing<br>Analysis of RNA secondary structure<br>Combined with RNase A to remove RNA<br>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<br>Remove mRNA before synthesizing the second strand of cDNA<br>Removal of the poly(A) sequence from mRNA after oligo(dT) hybridization |
| **MazF** | Single-stranded RNA | Unmodified ACA | mRNA analysis |

# Ordering Information

| Classification | REF No. | Name | Specs |
|---|---|---|---|
| **Template Construction** | EG15124S | M-MLV GIII Reverse Transcriptase | 10000 U |
| | EG24110S | 2× S705 HiFi Master Mix | 1 ml |
| | EG24110M | 2× S705 HiFi Master Mix | 5×1 ml |
| | EG24110L | 2× S705 HiFi Master Mix | 20×1 ml |
| | EG15205S | T4 DNA Ligase (Fast) | 1000 U |
| | EG25208S | Ultra T4 DNA Ligase | 1000 U |
| | EG24204S | DNA Assembly Mix Ultra | 50 rxns |
| | EG25202S/M | DNA Assembly Mix Mono | 50 rxns/250 rxns |
| | EG25202M | DNA Assembly Mix Mono | 250 rxns |
| | EG25207S | Golden Gate Assembly Kit (BsmBI) | 50 rxns |
| | EG25209S | Golden Gate Assembly Kit (BpiI) | 50 rxns |
| | EG25104M/L | 2× Taq PCR Aurora Mix | 5×1 ml/100×1 ml |
| | EG25109S/M | phi29 II DNA Polymerase | 500 U/2500 U |
| | EG25203S/M | TelN Protelomerase | 250 U/1000 U |
| | EG20907S | dNTPs (10 mM each) | 1 ml |
| | EG20901S | dATP (100 mM) | 1 ml |
| | EG20902S | dTTP (100 mM) | 1 ml |
| | EG20903S | dCTP (100 mM) | 1 ml |
| | EG20904S | dGTP (100 mM) | 1 ml |
| | EG20905S | dUTP (100 mM) | 1 ml |

# Ordering Information

| Classification | REF No. | Name | Specs |
|---|---|---|---|
| Template Generation | GMP501S | BsaI, GMP Grade | 20000 U |
| | GMP502S | XbaI, GMP Grade | 20000 U |
| | GMP503S | BspQI, GMP Grade | 10000 U |
| | GMP504S | XhoI, GMP Grade | 20000 U |
| | GMP505S | NheI, GMP Grade | 20000 U |
| | EG21504S | Esp3I(BsmBI), ADCF | 2000 U |
| | EG21504H | Esp3I(BsmBI), ADCF | 2000 U |
| | EG22504S | EcoRI, ADCF | 5000 U |
| | EG24601S | BsaI ELISA Kit | 96 T |
| | EG24602S | XhoI ELISA Kit | 96 T |
| | EG24603S | XbaI ELISA Kit | 96 T |
| | EG24604S | BsmBI ELISA Kit | 96 T |
| *In vitro* Transcription | EG24104S | T7 High Yield RNA Synthesis Kit | 50 rxns |
| | GMP101S | T7 RNA Polymerase, GMP Grade | 20000 U |
| | GMP101M | T7 RNA Polymerase, GMP Grade | 200000 U |
| | EG20124S | T7 RNA Polymerase | 5000 U |
| | EG20124M | T7 RNA Polymerase | 25000 U |
| | EG20125S | High T7 RNA Polymerase | 5000 U |
| | EG20125M | High T7 RNA Polymerase | 25000 U |
| | EG21916S | ATP (100 mM) | 1 ml |
| | EG21917S | CTP (100 mM) | 1 ml |
| | EG21918S | GTP (100 mM) | 1 ml |
| | EG21919S | UTP (100 mM) | 1 ml |
| | EG21920S | NTP Bundle (25 mM each) | 1 ml |
| | EG21921S | N1-Methyl-Pseudo-UTP (100 mM) | 100 µl |
| | EG21922S | Pseudo-UTP (100 mM) | 200 µl |
| | GMP102S | Murine RNase Inhibitor, GMP Grade | 40000 U |
| | EG20002S | RNase Inhibitor, Murine | 10000 U |
| | GMP105S | DNase I-ST, GMP Grade | 2000 U |
| | EG24202S | DNase I-ST, RNase-free | 1000 U |
| | EG24206S | DNase I-ST, RNase-free (Lyophilized) | 1000 U |
| | GMP103S | Pyrophosphatase, Inorganic (Yeast, GMP Grade) | 100 U |
| | EG23109S | Pyrophosphatase, Inorganic (Yeast) | 10 U |
| | EG23109M | Pyrophosphatase, Inorganic (Yeast) | 100 U |
| RNA Capping | EG22909S | Cap1 analogue AG | 100 µl |
| | EG25108S | Poly(A) RNA Polymerase | 100 U |
| | EG25108M | Poly(A) RNA Polymerase | 500 U |
| RNA Analysis | EG20003S | RNase A | 100 mg |
| | EG20003M | RNase A | 1 g |
| | EG24210S | RNase T1 | 100 KU |
| | EG24210M | RNase T1 | 5×100 KU |
| | EG24211S | Thermostable RNase T1 | 100 KU |
| | EG24211M | Thermostable RNase T1 | 5×100 KU |
| | EG20208S | RNase H | 1000 U |
| | EG24201S | Thermostable RNase H | 200 U |
| | EG24505S | MazF | 1000 U |
| | EG25521S | RNase GG | 2500 U |
| | EG25521M | RNase GG | 12500 U |

百 时 美
BestEnzymes

ISO 13485

WeChat Official Account

Research Subscription Account

WeChat Channels


**BestEnzymes Biotech Co., Ltd.**

Add: No.17 Huaguoshan Avenue, Lianyungang City, Jiangsu Province, China
Tel: 0518-8558 6628 · support@best-enzymes.com · http://en.best-enzymes.com


ver.202603