T7 RNA Polymerase, GMP Grade

REF: GMP101-S/M

Storage and Transportation Condition

Store at -20 ± 5℃ , Valid for 24 months. Transport at ≤ 0℃ .

Components

Component	GMP101S	GMP101M
T7 RNA Polymerase, GMP Grade (200 U/μl)	100 μl	1 ml
10× T7 RNA Pol Buffer, GMP Grade	1 ml	4×1 ml

Description

T7 RNA Polymerase, GMP Grade is derived from recombinant expression in *E.coli*. It is a DNA-dependent RNA polymerase that exhibits high specificity for the promoter sequence of bacteriophage T7. T7 RNA Polymerase utilizes double-stranded DNA templates containing the T7 promoter sequence and NTPs as substrates to synthesize single-stranded RNA complementary to the downstream of the promoter.

This product is manufactured and quality-controlled in compliance with GMP specifications, ensuring full traceability of the production process and raw materials. The entire production process does not involve the use of antibiotics or any animal-derived materials and excipients. Stringent controls are implemented for process-related impurities including host proteins, exogenous DNA, non-specific endonucleases, DNase, RNase, as well as microbial limits and bacterial endotoxins. This product meets the requirements for raw materials in fields such as vaccine and pharmaceutical production.

Definition of Activity Unit

One unit is defined as the amount of enzyme that incorporate 1 nmol of ATP into acid-insoluble material in 1 hour at 37℃ .

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction in T7 RNA Pol Buffer, GMP Grade containing 200 ng of supercoiled plasmid and 200 U of this product incubated for 4 hours at 37℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction in T7 RNA Pol Buffer, GMP Grade containing 15 ng of dsDNA fragments and 200 U of this product incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction in T7 RNA Pol Buffer, GMP Grade containing 500 ng of RNA and 200 U of this product incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Host Cell DNA

Using the third method of qPCR specified in General Chapter 3407 of ChP(2025) Volume IV, the residual *Escherichia coli* host cell DNA content of this product is below 10 copies/100 U.

Host Cell Protein

Using the method specified in General Chapter 3412 of ChP(2025) Volume IV, the residual *Escherichia coli* host cell protein content of this product is below 50 ppm.

Microbial Limit

Using the method specified in General Chapter 1105 of ChP(2025) Volume IV, the total aerobic microbial count of this product is below 5 cfu/ml, and the total combined yeasts/molds count is below 5 cfu/ml.

Bacterial Endotoxin

Using the first method of gel-clot specified in General Chapter 1143 of ChP(2025) Volume IV, the residual bacterial endotoxin content of this product is below 0.1 EU/KU.

Mycoplasma

Using a Mycoplasma detection kit (LAMP method) to test 200 U of this product, the result was negative.

Heavy Metals

Using the first method specified in General Chapter 0821 of ChP(2025) Volume IV, the residual heavy metals content of this product is below 10 ppm.

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Protocol

1. In vitro transcription

① Prepare the following reaction mixture on ice:

Reagent	Volume	Range of adjustment	Range of final concentrations
10× T7 RNA Pol Buffer, GMP Grade	2 μl	2 μl	1×
T7 RNA Polymerase, GMP Grade (200 U/μl)	1 μl	1~2 μl	10~20 U/μl
Pyrophosphatase, Inorganic (yeast, GMP Grade) (0.1 U/μl)	1 μl	0~1 μl	0~5 mU/μl
Murine RNase Inhibitor, GMP Grade (40 U/μl)	1 μl	0.5~2 μl	1~4 U/μl
DNA Template	1 μg	0.5~2 μg	25~100 ng/μl
ATP/CTP/GTP/UTP (100 mM each)[a,b]	2 μl each	1~2 μl each	5~10 mM each
Nuclease-Free Water	up to 20 μl	up to 20 μl	-

a. It is recommended to add Nuclease-Free Water first, followed by CTP/GTP/ATP/UTP.
b. Modified NTPs can be used to replace NTPs.

② Mix gently and spin down, then incubate at 37℃ for 2 h. If the length of transcript is less than 300 nucleotides, the reaction time can be extended to 3~16 hours.

③ After the in vitro transcription (IVT) reaction, add 1~2 U of DNase I-ST to the product and incubate at 37℃ for 15 minutes to remove the DNA template.

④ The purified mRNA obtained after quality inspection can be used for subsequent experiments or processes.

2. In vitro co-transcription

① Prepare the following reaction mixture on ice:

Reagent	Volume	Range of adjustment	Range of final concentrations
10× T7 RNA Pol Buffer, GMP Grade	2 μl	2 μl	1×
T7 RNA Polymerase, GMP Grade (200 U/μl)	1 μl	1~2 μl	10~20 U/μl
Pyrophosphatase, Inorganic (yeast, GMP Grade) (0.1 U/μl)	1 μl	0~1 μl	0~5 mU/μl
Murine RNase Inhibitor, GMP Grade (40 U/μl)	1 μl	0.5~2 μl	1~4 U/μl
DNA Template	1 μg	0.5~2 μg	25~100 ng/μl
ATP/CTP/GTP/UTP (100 mM each)[a,b]	2 μl each	1~2 μl each	5~10 mM each
Cap1 Analogue (100 mM)[c]	1.6 μl	0.8~1.6 μl	4~8 mM
Nuclease-Free Water	up to 20 μl	up to 20 μl	-

a. It is recommended to add Nuclease-Free Water first, followed by CTP/GTP/ATP/UTP.
b. Modified NTPs can be used to replace NTPs.
c. The molar ratio of cap analog to each NTP should be 4:5.

② Mix gently and spin down, then incubate at 37℃ for 2~3 h. If the length of transcript is less than 300 nucleotides, the reaction time can be extended to 4~16 hours.

③ After the in vitro transcription (IVT) reaction, add 1~2 U of DNase I-ST to the product and incubate at 37℃ for 15 minutes to remove the DNA template.

④ The purified mRNA obtained after quality inspection can be used for subsequent experiments or processes.

Notice

1. There can be significant differences in transcription efficiency for different template. In the initial experiments, it is recommended to start with the suggested amounts and then explore the optimal system within the range of adjustment.

2. The template DNA can be obtained by linearizing circular plasmids or PCR. The upstream region of the template DNA should contain the T7 promoter sequence and the downstream region should be blunt-ended or have a 5' overhang on the template strand. The purity of template DNA is crucial for in vitro transcription reactions, and residual RNase A introduced during plasmid DNA extraction can significantly affect the quality of transcribed RNA. It is recommended to use high-purity, RNase-free templates with an A_{260}/A_{280} ratio of 1.8~2.0.

3. Since all enzyme solutions contain glycerol, it is recommended that the total volume of enzyme added should not exceed 1/5 of the total reaction volume.

4. The co-transcription reaction rate is generally 1/5 to 1/2 of that of regular in vitro transcription.

5. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.

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