T7 Turbo RNA Polymerase (200 U/μl, GMP Grade)

GMP4120PB

Animal-free, Ampicillin-free

Vazyme

Instruction for Use

Version 24.2

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

T7 Turbo RNA polymerase (T7 RNAP), a protein encoded by the bacteriophage T7 DNA expressed in the recombinant *E. coli*, Clone number D05, is a DNA-dependent 5'→ 3' RNA polymerase that highly specifically recognizes T7 promoter sequences. This product uses single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) containing T7 promoter sequences as the template and NTPs as the substrate to synthesize RNA complementary to the ssDNA or dsDNA template strand downstream of the promoter. Compared with wild-type T7 RNA Polymerase, T7 Turbo RNA Polymerase effectively reduces the content of dsRNA, a by-product produced during transcription.While its co-transcription reaction product also has a higher capping rate.

This product is a GMP grade recombinant T7 RNA Polymerase mutant. Throughout the production process, strict control is exercised over process-related impurities such as host proteins, exogenous DNA, RNase, as well as microbial limits and bacterial endotoxins. Ampicillin and any animal-derived raw materials and excipients are not used or added in the entire production process. GMP-compliant production and quality management standards are adopted, ensuring traceability of the production process and raw materials and excipients, and meeting the requirements for raw materials and excipients in mRNA vaccine production and other related fields.

02/Product Components

Product Number	**GMP4120PB-01**	**GMP4120PB-02**	**GMP4120PB-03**
Product Specification	1 ml	5 ml	20 ml

* Related product: 10 × Transcription Buffer (GMP Grade) (Vazyme #GMP4101R)

03/Storage Conditions

Store at -20 ± 5℃ and transport at ≤0℃.

04/Product Information

Product Name	T7 Turbo RNA Polymerase (200 U/μl, GMP Grade)
Source	Recombinant *E. coli*
Activity	200 U/μl
Unit Definition	One unit of activity is defined as the amount of enzyme required to incorporate 1 nmol of [^{3}H] ATP into acid-insoluble precipitates within 1 hour at 37℃, pH 8.0.
Optimum Temperature	37℃
Storage Buffer	50 mM Tris-HCl (25℃, pH 7.9), 100 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 0.1% Triton X-100, 50% Glycerol
Storage Conditions	-20 ± 5℃, avoid repeated freeze-thaw cycles.

05/Application

1. Synthesize single-stranded RNA ranging from 10 to 14,000 nt, including mRNA, siRNA, gRNA and other RNA or precursors.
2. This product is suitable for in vitro transcription scenarios that cannot be satisfied by T7 RNA Polymerase (50 U/μl, GMP Grade), such as co-transcription sequences and difficult-to-transcribe sequences.

06/Quality Standards

Items	Inspection Methods	Standards
Properties	visual inspection	Clarified liquid
pH value	Ch.P2020 Part IV General rule 0631 pH determination method	7.3 - 8.3
Activity	In vitro transcription fluorescence method	≥200 U/μl
Purity	Ch.P2020 Part IV General 0512 High Performance Liquid Chromatography	≥95%
DNA endonuclease	150 U of the product was incubated with the pUC19 plasmid for 4 h at 37°C	Degradation ≤10%
DNA exonuclease	150 U of the product was incubated with the DNA substrate for 16 h at 37°C	Degradation ≤10%
RNase	RNase fluorescence assay kit method	Negative
Exogenous DNA	Ch.P2020 Part IV General Regulation 3407 Method III for the determination of exogenous DNA residues Quantitative PCR method	<120 fg/μl
Host Protein	Ch.P2020 Part IV General Regulation 3412 Determination of Protein Residues in Escherichia coli Bacteriophages	<50 ppm
Mycoplasmas	Mycoplasma assay kit method	Negative
Heavy metal residue	Ch.P2020 Part IV General Regulation 0821 Heavy Metals Inspection Act I	≤10 ppm
Bacterial Endotoxin	Ch.P2020 Part IV General 1143 Bacterial endotoxin assay Method 2 Photometric assay	<2 EU/ml
Microbiological Limit	Ch.P2020 Part IV General 1105 Microbiological Limit Checks for Non-sterile Products: Microbial Enumeration Methods	The total number of aerobic bacteria should be ≤5 cfu/ml. The total number of molds and yeasts should be ≤5 cfu/ml.

07/Experiment Process

1. Thaw all the kit components on ice, mix and pulse-spin in microfuge to collect solutions to bottom of tubes. Keep on ice.
2. Prepare a suitable reaction system according to the technical route.

2.1 In vitro transcription

Item	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer (GMP Grade)	2 μl	2 μl	1 ×
T7 Turbo RNA Polymerase (200 U/μl, GMP Grade)	2 μl	0.5 - 3 μl	5 - 30 U/μl
Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 5 mU/μl
Murine RNase Inhibitor (40 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 2 U/μl
ATP/CTP/GTP/UTP Solution (100 mM)	Each 2 μl	Each 1 - 2 μl	Each 5 - 10 mM
Template DNA	1 μg	0.5 - 2 μg	25 - 100 ng/μl
RNase-free ddH_2O	Up to 20 μl	Up to 20 μl	-

2.2 In vitro co-transcription

Item	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer (GMP Grade)	2 μl	2 μl	1 ×
T7 Turbo RNA Polymerase (200 U/μl, GMP Grade)	2 μl	1 - 3 μl	10 - 30 U/μl
Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 5 mU/μl
Murine RNase Inhibitor (40 U/μl, GMP Grade)	1 μl	0 - 1 μl	0 - 2 U/μl
CAG Trimer (100 mM, GMP Grade)	1.2 μl	0.5 - 1.8 μl	2.5 - 9 mM
ATP/CTP/GTP/UTP Solution (100 mM)	Each 1.5 μl	Each 1 - 2 μl	Each 5 - 10 mM
Template DNA	1 μg	0.5 - 2 μg	25 - 100 ng/μl
RNase-free ddH_2O	Up to 20 μl	Up to 20 μl	-

3. After sufficient mixing, the reaction conditions are: 37℃, 2 h.
4. Add 1μl of DNase I (1 U/μl, GMP Grade) (Vazyme #GMP4104PC) to the reaction system, mix and centrifuge them, and incubate them at 37℃ for 15 min to degrade template DNA.
5. The synthesised RNA can be used for subsequent experiments or processes after purification and quality control.

08/Notes

1. In vitro transcription

1.1 The recommended system is suitable for initial experiments with new sequences, as there are several-fold or even 10-fold differences in the reaction rates of different sequences.

1.2 It is recommended to set up a T7 RNA Polymerase dosage gradient under the recommended system to confirm the appropriate dosage for specific incubation conditions.

1.3 The amount of NTPs determines the yield plateau of the system. Under the conditions of balanced ratio of the four bases and sufficient amount of T7 RNA Polymerase, the yield plateau of each 10 mM (final concentration) amount of NTPs is 180 - 240 μg/20 μl.

1.4 The enzyme products contain glycerol, and it is recommended that the total volume of enzyme products added to the system should not exceed 1/5 of the reaction volume, and it is recommended to freeze and thaw no more than 7 times during use.

1.5 Template DNA can be obtained by post-fermentation linearisation or PCR amplification; RNase A residues introduced during plasmid DNA extraction can significantly affect the quality of the transcribed RNA, and it is recommended to use a high purity RNase-free plasmid with an OD260/280 of 1.8 - 2.0.

1.6 The yield is directly proportional to the reaction time. In cases where the recommended reaction conditions do not achieve the desired yield, extending the reaction time can be chosen to reach the target yield plateau. The reaction time can be adjusted within a range of 0-4 hours based on specific requirements.

1.7 Natural RNA can be quantified using a UV spectrophotometer; some modified nucleosides affect UV-absorbed light resulting in quantitation results that differ from those of natural nucleosides, which can be more accurately quantified using the fluorescence detection reagent RNA Quant assay kit (Vazyme#DD3511).

1.8 Product-related impurities dsRNA can be quantified using the dsRNA (Modified) Quantification Kit (ELISA) 2.0 (Vazyme #DD3509).

1.9 This in vitro transcription procedure yields uncapped RNA with a 5' triphosphate structure that cannot mediate eukaryotic translation. To obtain mRNA with Cap1 structure, use Vaccinia Capping Enzyme (10 U/μl, GMP Grade) (Vazyme #GMP4109PC) and mRNA Cap 2'-O-Methyltransferase (50 U/μl, GMP Grade) (Vazyme #GMP4110PC) for in vitro capping; Cap1 mRNA can also be obtained in one step by referring to "2.2 In vitro co-transcription".

2. In vitro co-transcription

2.1 The in vitro co-transcriptional initiation sequence needs to match the base type of the cap analog, generally using “AG” as the transcription initiation sequence to achieve higher capping efficiency, paired with the corresponding natural cap analog CAG Trimer (100 mM, GMP Grade) (Vazyme #GMP4118PC).

2.2 Due to differences in transcription initiation mechanisms, the in vitro co-transcriptional rate is generally 1/2 - 1/5 of the in vitro transcription rate. The recommended in vitro co-transcriptional system can be followed to achieve the desired reaction rate.

2.3 The amount of cap analog input for the recommended system usually yields mRNAs with >90% cap rate.

2.4 For the rest of the precautions, see "1. In vitro transcription".

09/Related Products

Product Number	Product Name
GMP4101R	10 × Transcription Buffer (GMP Grade)
GMP4102PA	Murine RNase Inhibitor (40 U/μl, GMP Grade)
GMP4103PC	Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)
GMP4104PC	DNase I (1 U/μl, GMP Grade)
GMP4109PC	Vaccinia Capping Enzyme (10 U/μl, GMP Grade)
GMP4110PC	mRNA Cap 2'-O-Methyltransferase (50 U/μl, GMP Grade)
GMP4118PC	CAG Trimer (100 mM, GMP Grade)
GMP4119PC	CAG Trimer (3'-OMe) (100 mM, GMP Grade)
DD3509	dsRNA (modified) quantification assay kit (ELISA) 2.0
DD3511	RNA Quant assay kit

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