T7 RNA Polymerase Variants Toolbox

DD4126

Vazyme

Instruction for Use

Version 25.1

Vazyme

Contents

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

01/Product Description

The T7 RNA Polymerase Variants Toolbox is specifically designed for in vitro RNA synthesis experiments, providing efficient and flexible solutions tailored to different sequences and application scenarios. The product includes four functionally specialized variants of T7 RNA polymerase and a universal transcription buffer. Users can flexibly combine different T7 RNA polymerases according to their specific needs, enhancing key performance indicators such as capping efficiency, product integrity, thermal stability, and dsRNA generation, thereby meeting the customized requirements for high-quality mRNA in vaccine development, gene therapy, and circular RNA synthesis research.

All four T7 RNA Polymerase, variant proteins encoded by the bacteriophage T7 DNA expressed in the recombinant *E. coli,* are DNA-dependent 5'→ 3' RNA polymerase that highly specifically recognizes T7 promoter sequences. These enzymes use 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 DNA template strand downstream of the promoter.

Compared to the wild-type T7 RNA polymerase：

T7 High-Capping RNA Polymerase (Clone number C10).

- Enhances co-transcriptional capping efficiency
- Adapting GG-Initiation (+1, +2) DNA template for co-transcriptional capping

T7 High-Integrity RNA Polymerase (Clone number P02).

- Improve the integrity of in vitro transcription products by 3 - 5%

T7 Thermostable RNA Polymerase (Clone number M1).

- Maintains catalytic activity at 50℃
- Suitable for circRNA synthesis with group I intron self-splicing and reduces dsRNA via high-temperature IVT.

T7 Low-dsRNA RNA Polymerase (Clone number D13).

- Reduce dsRNA byproducts to 5% compared to WT counterpart.

10 × Transcription Buffer.

- Optimized for in vitro transcription
- Compatible with all T7 variants in the toolbox
- Simplifies operational workflow

This product is for research scenarios only.

02/Components

Components	DD4126-01	DD4126-02	DD4126-03
T7 High-Capping RNA Polymerase (200 U/μl)	100 μl	1 ml	5 ml
T7 High-Integrity RNA Polymerase (200 U/μl)	100 μl	1 ml	5 ml
T7 Thermostable RNA Polymerase (200 U/μl)	100 μl	1 ml	5 ml
T7 Low-dsRNA RNA Polymerase (200 U/μl)	100 μl	1 ml	5 ml
10 × Transcription Buffer	1 ml	10 ml	50 ml

03/Storage

Store at -30 ~ -15℃ and ship at ≤0℃.

04/Applications

Synthesize single-stranded RNA ranging from 10 to 14,000 nt, including mRNA, siRNA, gRNA and other RNA or precursors.

05/Self-prepared Materials

Template: linearized plasmid with a T7 RNA polymerase promoter sequence, PCR product or synthetic DNA fragment.

In vitro transcription components: RNase Inhibitor, Inorganic Pyrophosphatase, natural and modified nucleotides, RNase-free ddH_2O.

Purification: phenol, chloroform, sodium acetate, ethanol; lithium chloride, EDTA, ethanol; magnetic beads for RNA purification; RNA purification column.

06/Notes

1. In vitro transcription
   a. 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.
   b. 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.
   c. 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.
   d. 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 OD_{260}/OD_{280} of 1.8 - 2.0.

e. 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 h based on specific requirements.

f. Product-related impurities dsRNA can be quantified using the EasyAna dsRNA (Modified) Quantitative Detection Kit (ELISA) 2.0 (Vazyme #DD3509).

g. 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. In vitro co-transcription".

2. In vitro co-transcription

a. 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.

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

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

07/Experiment Process

T7 High-Capping RNA Polymerase

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.

In vitro co-transcription

Components	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer	2 μl	2 μl	1 ×
T7 High-Capping RNA Polymerase (200 U/μl)	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)	0.4 μl	0.1 - 1.8 μl	0.5 - 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	-

▲ In vitro co-transcription initiation sequences usually need to match the base types of cap analogs. Using T7 High-Capping RNA Polymerase allows for the use of "GG" as the transcription initiation sequence and achieves high capping efficiency, in combination with the specific buffer and the cap analog.

3. After sufficient mixing, the reaction conditions are: 37℃, 2 h.
4. Add 1 μl 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.

T7 High-Integrity RNA Polymerase/T7 Low-dsRNA RNA Polymerase

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.

In vitro transcription

Components	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer	2 μl	2 μl	1 ×
T7 RNA polymerase (200 U/μl)	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	-

In vitro co-transcription

Components	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer	2 μl	2 μl	1 ×
T7 RNA polymerase (200 U/μl)	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 1 μl 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.

T7 Thermostable RNA Polymerase

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.

One-step synthesis of circular RNA

Components	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer	2 μl	2 μl	1 ×
T7 Thermostable RNA Polymerase (200 U/μl)	2 μl	1 - 3 μl	10 - 30 U/μl
Pyrophosphatase, Inorganic (yeast, 0.1 U/μl, GMP Grade)	1 μl	0.5 - 2 μl	2.5 - 10 mU/μl
Murine RNase Inhibitor (40 U/μl, GMP Grade)	1 μl	0.5 - 2 μl	1 - 4 U/μl
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	-

▲ The modified group I intron self-splicing system is commonly used for in vitro circular RNA synthesis, and the one-step method for circRNA synthesis can be implemented using T7 Thermostable RNA Polymerase.

3. After sufficient mixing, the reaction conditions are: 48℃, 1.5 h.

▲ The change in temperature can directly affect the efficiency and specificity of in vitro transcription. The reaction temperature can be adjusted between 48 and 50℃ to enhance the cyclization efficiency. To prevent RNA products from degrading at high temperatures, the reaction time should not exceed 2 h.

4. Add 1 μl 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.

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