T7 High-Capping RNA Polymerase (200 U/μl)

DD4122-PB

Version 25.1

Animal-free, Ampicillin-free

Product Description

T7 High-Capping RNA Polymerase, a variant protein encoded by the bacteriophage T7 DNA expressed in the recombinant *E. coli*, 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 DNA template strand downstream of the promoter. Compared with wild-type T7 RNA Polymerase, T7 High-Capping RNA Polymerase can effectively enhance the capping rate of mRNA products in in vitro co-transcription.

This product is for research scenarios only.

Components

Component	DD4122-PB-01
T7 High-Capping RNA Polymerase (200 U/μl)	1 ml

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

Storage

Store at -30 ~ -15℃ and ship at ≤0℃.

Applications

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 difficult-to-cap co-transcriptional sequences.

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 High-Capping 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 dsRNA (Modified) Quantification 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. 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 natural cap analog CAG Trimer (100 mM, GMP Grade) (Vazyme #GMP4118PC) or modified cap analog CAG Trimer (3'-OMe) (100 mM, GMP Grade) (Vazyme #GMP4119PC).

b. 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.

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

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

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

Components	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer (GMP Grade)	2 μl	2 μl	1 ×
T7 High-Capping 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	-

2.2 In vitro co-transcription

Components	Recommended system	System scope adjustment	Final concentration
10 × Transcription Buffer (GMP Grade)	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	-

3. After sufficient mixing, the reaction conditions are: 37℃, 2 h.

4. Add 1 μl 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.

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)
GMP4120PB	T7 Turbo RNA Polymerase (200 U/ul, GMP Grade)
DD4123-PB	T7 High-Integrity RNA Polymerase (200 U/μl)
DD4124-PB	T7 Thermostable RNA Polymerase (200 U/μl)
DD4125-PB	T7 Low-dsRNA RNA Polymerase (200 U/μl)
DD3509	EasyAna dsRNA (Modified) Quantitative Detection Kit (ELISA) 2.0