T7 RNA Polymerase (HC)

REF: EG25107S

Storage and Transportation Condition

Store at -20℃ for 2 years.

Components

Component	Amount
T7 RNA Polymerase (HC) (200 U/μl)	100 μl
10× T7 RNA Pol Buffer	1.25 ml

Description

T7 RNA Polymerase 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.

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 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 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 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.

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	2 μl	2 μl	1×
T7 RNA Polymerase (HC) (200 U/μl)	1 μl	1~2 μl	10~20 U/μl
Pyrophosphatase, Inorganic (yeast) (0.1 U/μl)	1 μl	0~1 μl	0~5 mU/μl
Murine RNase Inhibitor (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	2 μl	2 μl	1×
T7 RNA Polymerase (HC) (200 U/μl)	1 μl	1~2 μl	10~20 U/μl
Pyrophosphatase, Inorganic (yeast) (0.1 U/μl)	1 μl	0~1 μl	0~5 mU/μl
Murine RNase Inhibitor (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.

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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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