T7 RNA Polymerase

Cat #: C-BSM0124

Size: 5000 U / 25000 U

Storage: -20°C

Components

Components	**C-BSM0124-5000U**	**C-BSM0124-25000U**
T7 RNA Polymerase (50 U/μl)	5000 U	25000 U
10×T7 RNA Polymerase Buffer	1.25 ml	1.25 ml

Description

Bacteriophage T7 RNA Polymerase is a DNA-dependent RNA polymerase that is highly specific for the T7 phage promoters. T7 RNA polymerase utilizes double-stranded DNA containing the T7 promoter sequence as a template and NTPs as substrates to synthesize RNA that is complementary to the downstream DNA of the promoter.

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of ATP into a polynucleotide fraction in 60 minutes at 37°C.

Application

1. Synthesis of single-stranded RNA, including precursors of mRNA, siRNA, gRNA, and other types of RNA.
2. Synthesis of labeled or unlabeled highly specific RNA probes.
3. Synthesis of capped mRNA using cap analogs.

Quality Control

Purification

Greater than 95% by SDS-PAGE.

Endonuclease residues

Incubate 20 U of T7 RNA Polymerase with supercoiled plasmid DNA at 37°C for 4 hours, no changes for the plasmid were detected in DNA electrophoresis.

DNase residues

Incubate T7 RNA Polymerase with double-stranded DNA at 37°C for 16 hours, no changes for the DNA were detected in DNA electrophoresis.

RNase residues

Incubate T7 RNA Polymerase with RNA at 37°C for 1 hour, no degradation for the RNA was detected in electrophoresis.

Method of application

Components	Amount	Final concentration
10× T7 RNA Polymerase Buffer	2 µl	1×
CTP / GTP/ ATP/ UTP (100 mM each)	0.1~0.4 µl each	0.5~2 mM each
RNase Inhibitor (40 U/µl)	0.5~1 µl	1~2 U/µl
Template DNA	0.1~1 µg	-
T7 RNA Polymerase(50 U/µl)	1~2 µl	-
Nuclease-Free Water	up to 20 µl	-

Note: It is recommended to add Nuclease-Free Water first and then add CTP/GTP/ ATP/ UTP.

Recommended reaction conditions

Incubate at 37°C for 1 hour. If the transcript length is less than 300 nt, the reaction time can be extended to 2-16 hours.

After the reaction, add 1 µl of dsDNase to the above 20 µl reaction mixture and incubate at 37°C for 15 minutes to remove the DNA template.

Notes

1.The purity of the template DNA is crucial for in vitro transcription. RNase A residues introduced during plasmid DNA extraction can significantly affect the quality of transcribed RNA. It is recommended to use high-purity RNase-free plasmids with an OD260/280 ratio of 1.8-2.0.

2.The template DNA can be obtained through linearized circular plasmids or PCR. The upstream region of the template DNA should contain the T7 promoter sequence, and the downstream region should have blunt ends or a 5' overhang on the coding strand.

3.For your safety and health, please wear lab coat, disposable gloves, and mask while performing the experiment.