Thermostable T7 RNA Polymerase

Cat #: C-BSM0125

Size: 5000 U / 25000 U

Storage: -20°C

Components

Components	C-BSM0125-5000U	C-BSM0125-25000U
Thermostable T7 RNA Polymerase (50 U/μl)	5000 U	25000 U
10×T7 RNA Polymerase Buffer	1.25 ml	1.25 ml

Description

Thermostable T7 RNA Polymerase is a genetically engineered heat-stable T7 RNA polymerase that is highly specific for the phage T7 promoter and can react at higher temperatures than wild-type phage T7 RNA polymerase. Thermostable T7 RNA Polymerase can be efficiently transcribed in vitro at 37~52°C.

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of ATP into a polynucleotide fraction in 60 minutes at 50°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 Thermostable 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 Thermostable 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 Thermostable T7 RNA Polymerase with RNA at 37°C for 1 hour, no degradation for the RNA was detected in electrophoresis.

Functional test

In vitro transcriptional synthesis experiments, the target bands can be detected by RNA 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	-
Thermostable 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 50°C for 1 hour.

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.