T7 RNA Polymerase

Cat.No.:HYJ211

Product Description

T7 RNA polymerase is a DNA-dependent RNA polymerase derived from the bacteriophage T7. It possesses highly specific 5'→3' RNA polymerase activity. T7 RNA polymerase is highly specific for the T7 promoter and can synthesize large amounts of RNA using the downstream sequence of the T7 promoter as a template.

Components

Components	5KU	50KU	500KU	5000KU
T7 RNA Polymerase (50 U/μL)	0.1 mL	1 mL	10 mL	100 mL
10× T7 Buffer	2 ×0.1 mL	2 ×1 mL	2 × 10 mL	2 × 100 mL

Storage

Transport below 0°C; store at -25 to -15°C. Valid for 24 months.

Storage buffer

50 mM Tris-HCl, 100 mM NaCl, 20mM β-ME, 1 mM EDTA, 50% Glycerol, 0.1% (w/v) Triton X-100, pH 7.9 @ 25°C

Activity Definition

One activity unit (U) is defined as the amount of enzyme required to catalyze the conversion of NTP to 1 nmol of PPi in a standard reaction system at 37°C within 1 hour.

Quality Control

1. **Endonuclease Residue Detection:** 50U of this enzyme and 1 μg of λDNA were added to a 50 μL reaction system and incubated at 37°C for 16 hours. The DNA bands on agarose gel electrophoresis remained unchanged.
2. **Exonuclease Residue Detection:** 50 U of this enzyme and 1 μg of λ-Hind III digested DNA were added to a 50μL reaction system and incubated at 37°C for 16 hours. The DNA bands on agarose gel electrophoresis remained unchanged.

3. **Nickase Residue Detection:** 50U of this enzyme and 1 μg of pBR322 DNA were added to a 50 μL reaction system and incubated at 37°C for 16 hours. The DNA bands on agarose gel electrophoresis remained unchanged.
4. **RNase residual test:** Add 50U of this enzyme and 1.6 μg of MS2 RNA to a 50 μL reaction system and incubate at 37°C for 16 hours. The RNA bands observed on agarose gel electrophoresis remain unchanged.
5. **Heat inactivation:** 75°C for 10 minutes.

Reaction system and conditions

Components	Volume
10× T7 Buffer	2 uL
ATP/GTP/CTP/UTP (100 mM each)	0.4 μL each (2 mM each Final)*
RNase inhibitors	1 μL (40 U)
Pyrophosphatase Inorganic	0.5 μL (0.05U)
T7 RNA Polymerase (50 U/μL)	1 μL
Linearized template DNA	1 μg
Nuclease-free water	Up to 20uL

Add the reaction components in the order listed above.

*10× T7 Buffer is only suitable for a final NTP concentration of 2mM. If a 7.5mM-10mM concentration is required, please use a high-yield kit.

Reaction time: Incubate at 37°C for 2 hours.

Termination reaction： Add 2 μL of 0.2 M EDTA (pH = 8.0 at 25°C) or heat at 75°C for 10 min.

DNA template removal: Treat with DNase I (2 U) at 37°C for 15 minutes.

Notes

1. The transcription reaction should be prepared in an RNase-free environment. Wearing gloves is recommended during the operation. Use nuclease-free water, pipette tips, and reaction tubes for the system.
2. RNA yield can be increased by increasing the NTP concentration (up to 10 mM each), and the Mg^{2+} concentration should also be appropriately increased.
3. The reaction system should be prepared at room temperature to avoid precipitation caused by the reaction of DNA with 10× T7 Buffer at 4°C.
4. Incomplete linearization of the template DNA may reduce the yield and length of the transcript.

5. The reaction volume can be scaled up or down according to actual needs.
6. If the reaction product decreases, add 20 mM fresh DTT to the reaction system.
7. For transcripts ≤500 bp, it is recommended to extend the transcription time to 4-8 hours.
8. 10× T7 Buffer is prone to precipitation after frozen storage. Before use, ensure complete dissolution. If precipitation occurs, reheat thoroughly at 37°C and shake to dissolve.
9. 10× T7 Buffer is only suitable for NTPs with a final concentration of 2mM. If a 7.5mM-10mM concentration is required, please use a high-yield kit.