T7 RNA Polymerase

T292917

Introduction:

T7 RNA Polymerase is a DNA-dependent RNA polymerase from T7 phage ， that possesses a strong and specific 5′→ 3′ RNA polymerase activity. T7 RNA Polymerase has high specificity for T7 promoter sequences and will synthesize large quantities of RNA from a DNA fragment inserted downstream from a promoter.

Transportation and Storage：

Transportation under 0℃ and storage at -20℃

Unit Definition：

One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into an acid-insoluble material in 1 hour at 37°C.

Quality Control Assays：

1. Endonuclease Activity: Incubation of a 40 μL reaction containing a minimum of 200 U of T7 RNA Polymerase with 4 μg pUC19 DNA for 16 hours at 37℃ results in no detectable degradation as determined.
2. Exonuclease Activity: Incubation of a 50 μL reaction containing a minimum of 200 U of T7 RNA Polymerase with 1 μg Hind III digest λ DNA for 16 hours at 37℃ results in no detectable degradation as determined.
3. Nickase Activity: Incubation of a 50 μL reaction containing a minimum of 200 U of T7 RNA Polymerase with 1 μg pBR322 DNA for 16 hours at 37℃ results in no detectable degradation as determined.
4. RNase Activity: Incubation of a 50 μL reaction containing a minimum of 200 U of T7 RNA Polymerase with 1.6 μg MS2 RNA for 4 hours at 37℃ results in no detectable degradation as determined.
5. Heat Inactivation: 75℃ for 10min.

RNA Synthesis Reaction:

Reagent	Amount
Nuclease-free water/DEPC	Up to 20 μL
10×T7 RNA Polymerase Buffer	2 μL
ATP/GTP/CTP/UTP (100mM each)	0.4 μL each (2mM each Final)
RNase inhibitor	1 μL (40 U)
Inorganic pyrophosphatase	0.5 μL (0.05 U)
T7 RNA Polymerase (50 U/μL)	1 μL
Linearized template DNA	1 μg

Incubation Time: 37℃ for 1-2 hours.

Stop of Reaction: Add 2 μL 0.2 M EDTA (pH=8.0@25℃) or heat to 75°C for 10min.

DNA Removal: DNA template can be removed with 2U DNase I (RNase-free) and incubation for 15 min at 37°C.

Inhibitors: Metal chelators, enzyme activity is reduced by 50% at NaCl or KCl concentration above 150 mM.

Matters needing attention:

1. The transcription reaction should be performed under contamination without RNases. Wearing gloves is advisable. The tips, tubes and water should be nuclease free. All the solutions should be made up in nuclease free water.

2. The RNA synthesis reaction mixture should be prepared at room temperature, since DNA may precipitate in the presence of spermidine at 4°C.

3. The yield of proper length transcripts decreases if the template DNA is incompletely linearized.

4. The reaction mixture can be scaled up or down.