

Code; RP6T7  
Size; 10,000 units



COSMO BIO Co., LTD.  
Inspiration for Life Science

# T7 RNA Polymerase (Recombinant)

## Supplied Reagents

- T7 RNA Polymerase
- 10 X T7 RNA Polymerase Buffer

**Concentration** : 50 units/  $\mu$ L

**Storage** : -20 °C

**Description** : T7 RNA Polymerase catalyzes the synthesis of RNA in the presence of the DNA template including T7 phage promoter.

## Storage Buffer :

20 mM Potassium Phosphate (pH7.7)  
100 mM NaCl  
1 mM DTT  
1 mM EDTA  
50 % Glycerol

## 10 X T7 RNA Polymerase reaction buffer :

400 mM Tris-HCl (pH8.0)  
200 mM NaCl  
60 mM MgCl<sub>2</sub>  
20 mM Spermidine  
100 mM DTT

**Source** : Recombinant protein, expressed in *E.coli*.

## Purity :

> 95%, as determined by SDS-PAGE visualized by CBB stain.

## Absence of endonucleases :

Incubation of 250 units of enzyme with 1  $\mu$ g  $\lambda$  DNA-Hind III fragments for 16 hours at 37°C in reaction buffer gave no detectable banding pattern or degradation of  $\lambda$  DNA- DNA-Hind III fragments.

## Absence of RNases :

Incubation of 250 units for 5 hours at 37°C in reaction buffer with 2  $\mu$ g 16S, and 23S rRNA resulted in no detectable degradation of the RNA.

## Unit definition :

One unit of RNA polymerase catalyzes the incorporation of 1nmole of a labeled ribonucleoside triphosphate into RNA in 1 hour at 37°C and pH 8.0 using a DNA template with the T7 promoter.

## Applications

- Synthesis of single-strand RNA
- Synthesis of labeled RNA probe
- Synthesis of precursors of siRNA.

## Standard Application :

- A) Reagents to be supplied by user
- template DNA including T7 promoter
  - Ribonucleoside triphosphates
  - Nuclease-Free Water
  - RNase inhibitor (Optional)

## B) Synthesis of single-stranded RNA

1. Prepare the following reaction mixture in a sterile microcentrifuge tube.

10 X T7 RNA Polymerase buffer	5 $\mu$ L
NTP	each 0.5mM
Template DNA	1 $\mu$ g
T7 RNA Polymerase (50 units/ $\mu$ L)	1 $\mu$ L
Nuclease-Free Water	up to 50 $\mu$ L
RNase inhibitor	Optional

2. Incubate at 37°C for 1 hour

## References :

- 1) Davanloo, P. *et al.*, *Proc. Natl. Acad. Sci. USA*, 81, 2035 (1984).
- 2) Zawadzki, V. and Gross, H.J., *Nucleic Acids Research*, 19, 1948 (1991).

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