T7 RNA Polymerase

T7 RNA Polymerase (20u/ul)	250 µl
5X Transcription Buffer	1.25 ml

For research use only
Cat No: YT9070
Size: 5000 U
Store at –20°C

Bacteriophage T7 RNA Polymerase is a DNA dependent RNA polymerase that is routinely used to produce RNA sequences from DNA templates . The enzyme catalyzes the 5'→3' synthesis of RNA on either single-stranded DNA or double-stranded DNA downstream from the promoter with extremely high specificity for the T7 promoter. T7 RNA Polymerase also can use modified nucleotides(e.g., biotin-, digoxigenin-, fluorescein-labeled nucleotides) as substrates for RNA synthesis.

Applications:

- In vitro Synthesis of RNA
- Synthesis of highly radiolabeled RNA probes
- Synthesis of RNA as hybridization probes
- RNA synthesis for in vitro translation and RNA splicing studies
- In studies of RNA secondry structure and RNA-protein interactions

Features

- RNA synthesis from DNA template
- High specificity for the T7 promotor

SOURCE
E. coli strain that carries the cloned T7 RNA polymerase gene from T7 phage.

Molecular Weight : 99 kDa monomer

Definition of Activity Unit :
One unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction in 60 minutes at 37 °C.

Storage Buffer:
20 mM Tris-HCl (pH 7.5), 200mM NaCl , 0.25 mM EDTA , 0.01% NP-40 , 2.5 mM DTT , 50% Glycerol

5X Transcription Buffer
200mM Tris-HCl (pH 8.0), 100mM MgCl2 , 12.5mM TCEP , 10mM spermidine

Inhibition and Inactivation
• Inhibitors: metal chelators, enzyme activity is reduced by 50% at NaCl or KCl concentration above 150 mM.
• Inactivated by heating at 70 °C for 10 min or by addition of EDTA.

Protocol for in vitro transcription

1. Linearize template DNA with a restriction enzyme. Extract DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in DEPC-treated Water.
2. Prepare the following reaction mixture:

5X Transcription buffer	10µl
ATP/GTP/CTP/UTP Mix,10Mm each	10µl (2mM final concentration)
Linear template DNA	1µg
RNase Inhibitor	1.25µl (50U)
T7 RNA Polymerase	30U
DEPC-treated Water	to 50µl

3. Incubate at 37 °C for 2 hours.
4. Optional: To remove template DNA add 2 µL (2 U) of DNase I, RNase-free, mix and incubate at 37 °C for 15 min.
5. Inactivate DNase I by phenol/chloroform extraction.

Note

- The transcription reaction should be performed under conditions that exclude contamination with RNases. The tips, tubes and water should be nuclease free. All the solutions should be made up in nuclease free water.
- Prepare the reaction mixture at room temperature.
- Under the conditions described above, more than 10 µg RNA per 1 µg template DNA is obtained.
- Notice that if the DNA template incompletely linearized, the yield of proper length transcripts decreases.