T7 RNA Polymerase

1-800-632-7799
info@neb.com
www.neb.com

M0251S 0091208140 81

M0251S

5,000 units **50,000 U/ml** **Lot: 0091208**

RECOMBINANT **Store at –20°C** **Exp: 8/14**

Description: Initiation of transcription with T7 RNA Polymerase is highly specific for the T7 phage promoters. Cloning vectors have been developed which direct transcription from the T7 promoter through polylinker cloning sites (1). These vectors allow *in vitro* synthesis of defined RNA transcripts from a cloned DNA sequence. Under optimal conditions, greater than 700 moles of T7 RNA transcript can be synthesized per mole of DNA template (2). RNA produced using the T7 Polymerase is biologically active as mRNA (3) and can be accurately spliced (4). Anti-sense RNA, produced by reversing the orientation of the cloned DNA insert, has been shown to specifically block mRNA translation *in vivo* (5).

Labeled single-stranded RNA transcripts of high specific activity are simple to prepare with T7 RNA Polymerase (6). Increased levels of detection in nucleic acid hybridization reaction can also be obtained due to the greater stability of RNA:DNA hybrids with respect to RNA: RNA or DNA:DNA hybrids (7).

Source: Isolated from *E. coli* BL21 carrying the plasmid pAR1219 which contains T7 gene I under the control of the inducible *lac* UV5 promoter (9).

Applications:

- Radiolabeled RNA probe preparation (6)
- RNA generation for *in vitro* translation (10)
- RNA generation for studies of RNA structure, processing and catalysis (10)
- Expression control via anti-sense RNA

Supplied in: 100 mM NaCl, 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 20 mM 2-mercaptoethanol, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
10X RNAPol Reaction Buffer.

Reaction Conditions: 1X RNAPol Reaction Buffer, supplemented with 0.5 mM each ATP, UTP, GTP, CTP and DNA template containing the T7 RNA Polymerase promoter. Incubate at 37°C.

1X RNAPol Reaction Buffer:
40 mM Tris-HCl
6 mM $MgCl_2$
2 mM spermidine
10 mM dithiothreitol
pH 7.9 @ 25°C

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.

Unit Assay Conditions: 1X RNAPol Reaction Buffer, supplemented with 0.5 mM each ATP, UTP, GTP, CTP and 1 µg T7 DNA in 50 µl.

Conditions For Making RNA Probes:
1X RNAPol Reaction Buffer
100 µg/ml BSA
500 µM each of CTP, UTP, GTP and ATP
1000 units/ml T7 RNA Polymerase
100 µg/ml of DNA template incubated at 37°C (6).

For RNA of high specific activity, the concentration of the radioactive nucleotide should be made limiting (12 µM) (6).

To protect RNA products against the inadvertent presence of ribonuclease in the reaction mixture, ribonuclease inhibitor should be added to a final concentration of 1 µ/µl.

Specific Activity: 1,200,000 units/mg.

Quality Assurance: T7 RNA Polymerase is purified free of other RNA polymerases, DNases and RNases.

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Quality Control Assays

16-Hour Incubation: Incubation of 200 units of enzyme with 1 µg λ DNA at 37°C in 50 µl reaction buffer gave no detectable banding pattern or degradation of λ DNA.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

RNase Activity: A 10 µl reaction in RNA polymerase buffer containing 40 ng of 300 base single-stranded RNA transcript and 200 units of T7 RNA polymerase incubated 2 hours at 37°C resulted in less than 30% degradation as determined by TBE-urea polyacrylamide gel electrophoresis.

Exonuclease Activity: Incubation of 100 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% acid soluble counts.

Nuclease Activity: After incubation of HindIII fragments of λ DNA with 200 units of Polymerase for 1 hour, > 90% of DNA fragments were ligated with T4 DNA Ligase (@ a 5´ termini concentration of 1–2 µM at 16°C). Of these ligated fragments Hind III recleaved > 90%.

Polymerase Specificity: Incubation of 200 units of enzyme in 50 µl assay buffer with λ DNA as template resulted in incorporation of < 1.5% of the amount incorporation when T7 DNA was used as a template.

Notes On Use: Dithiothreitol is required for activity.

T7 RNA Polymerase is extremely sensitive to salt inhibition. For best overall salt concentration should not exceed 50 mM.

Higher yields of RNA may be obtained by raising NTP concentrations (up to 4 mM each). Mg^{2+} concentration should be raised to 4 mM above the total NTP concentration. Additionally, inorganic pyrophosphatase should be added to a final concentration of 4 units/ml.

An apparent decrease in enzyme activity over time may be due to the breakdown of dithiothreitol in the reaction buffer; even when stored at –20°C. If you observe a decrease in yield, try supplementing your reactions with a final concentration of 10 mM fresh dithiothreitol.

References:

1. Schenborn, E.T. and Meirendorf, R.C. (1985) *Nucleic Acids Res.* 13, 6223–6236.
2 Noren, C.J. et al. (1990) *Nucleic Acids Res..* 18, 83–88.
3. Kreig, P.A. and Melton, D.A. (1984) *Nucleic Acids Res.* 12, 7057–7070.
4. Green, M.R., Maniatis, R. and Melton, D.A. (1983) *Cell* 32, 681–694.
5. Melton, D.A. et al. (1985) *Proc. Natl. Acad. Sci. USA* 82,144–148.
6. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning*: *A Laboratory Manual,* (2nd ed.), (pp. 10.27–10.37). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
7. Zinn, K. et al. (1983) *Cell* 34, 865–879.
8. Butler, E.T. and Chamberlin, J. (1982) *J. Biol. Chem.* 257, 5772–5778.
9. Davanloo, P. et al. (1984) *Proc. Natl. Acad. Sci. USA* 81, 2035–2039.
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