

SP6 RNA Polymerase



1-800-632-7799
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M0207S 015131015101

M0207S



2,000 units 20,000 U/ml Lot: 0151310
RECOMBINANT Store at -20°C Exp: 10/15

Description: Initiation of transcription with SP6 RNA Polymerase is highly specific for the SP6 phage promoter. Cloning vectors have been developed which direct transcription from the SP6 promoter through polylinker cloning sites (1). These vectors allow *in vitro* synthesis of defined RNA transcripts from a cloned DNA sequence. RNA produced using the SP6 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 SP6 RNA with Polymerase (6). Increased levels of detection in nucleic acid hybridization reactions can also be obtained due to the greater stability of RNA:DNA hybrids with respect to RNA:RNA or DNA:DNA hybrids (7).

Source: An *E. coli* strain that carries the cloned gene for SP6 RNA Polymerase from *Salmonella typhimurium* LT2Z.

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 SP6 RNA Polymerase promoter. **Incubate at 40°C.**

1X RNAPol Reaction Buffer:

40 mM Tris-HCl
6 mM MgCl₂
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 40°C.

Assay Conditions: 1X RNAPol Reaction Buffer, supplemented with 0.5 mM each ATP, UTP, GTP, CTP and 1 µg SP6 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
1,000 units/ml SP6 RNA Polymerase
100 µg/ml of DNA template incubated at 40°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 mix, ribonuclease inhibitor should be added to a final concentration of 1 µl/µl.

Quality Assurance: SP6 RNAPol Polymerase is purified free of other RNA polymerases, DNases and RNases.

Quality Control Assays

16-Hour Incubation: 200 units with 1 µg λ DNA in 50 µl assay buffer at 40°C gave no detectable banding pattern or degradation of λ DNA.

Endonuclease Activity: Incubation of 200 units of SP6 RNA Polymerase with 1 µg of φX174 RF I DNA for 4 hours at 40°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

RNase Activity: Incubation of 200 units for 4 hours at 40°C in 50 µl assay buffer with 1 µg RNA Ladder (NEB #N0362) resulted in no detectable degradation of the RNA.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

CERTIFICATE OF ANALYSIS

Exonuclease Activity: Incubation of 200 units for 4 hours at 40°C in 50 µl assay buffer containing 1 µg sonicated [³H] DNA (10⁵ cpm/µg) gave < 0.1% acid soluble counts.

Nuclease Activity: After incubation of 1 µg Hind III fragments of λ DNA with 50 units of polymerase for 1 hour, > 95% of DNA fragments were ligated with T4 DNA Ligase (at a 5' termini concentration of 1–2 µM at 16°C). Of these ligated fragments Hind III recleaved > 95%.

Polymerase Specificity: Incubation of 200 units in 50 µl assay buffer with λ DNA as template resulted in incorporation of < 0.5% of the amount incorporated when SP6 DNA was used as a template.

Notes On Use: Dithiothreitol is required for activity.

SP6 RNA Polymerase is extremely sensitive to salt inhibition. For best results overall salt concentration should not exceed 50 mM.

SP6 RNA Polymerase is 30% more active at 40°C than at 37°C.

Higher yields of RNA may be obtained by raising NTP concentrations (up to 4 mM each). Mg²⁺ 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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