SP6 RNA Polymerase

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

M0207S 015150217021

M0207S

2,000 units **20,000 U/ml** **Lot: 0151502**

RECOMBINANT **Store at –20°C** **Exp: 2/17**

Description: Bacteriophage SP6 RNA Polymerase is a DNA-dependent RNA polymerase that is highly specific for the SP6 phage promoter. The 98.5 KD polymerase catalyzes *in vitro* RNA synthesis from a cloned DNA template under the SP6 promoter. RNA synthesized using the SP6 RNA Polymerase is suitable for many applications in research and biotechnology.

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

Applications:

- Radiolabeled RNA probe preparation
- Non-isotopic RNA labeling
- Preparation of RNA vaccines
- Guide RNA for gene targeting
- mRNA for *in vitro* translation and micro injection
- RNA structure, processing and catalysis studies
- RNA amplification
- Anti-sense RNA for gene expression experiment

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 37°C.

1X RNAPol Reaction Buffer:
40 mM Tris-HCl
6 mM $MgCl_2$
2 mM spermidine
1 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 DNA containing the SP6 promoter in 50 µl.

Protocol for Standard RNA Synthesis: Assemble the reaction at room temperature in the following order.

COMPONENTS	AMOUNT	CONCENTRATION
H_2O	X µl	
10X Reaction Buffer	2 µl	
NTP	X µl	0.5 mM each
Template DNA	X µl	0.2–1 µg
RNase Inhibitor (optional)	0.5 µl	1 U/µl final
Fresh DTT (optional)	X µl	5 mM final
SP6 RNA Pol	2 µl	
Total Volume	20 µl	

Incubate at 37°C for 1 hour. For shorter (< 300 nt) transcripts incubate at 37°C for 2–16 hours.

Notes on use:
For radio labeled high specific activity RNA probes, the concentration of the radioactive nucleotide should be limited to 6 µM.

To protect RNA against ribonuclease, RNase inhibitor (NEB #M0314 or #M0307) should be added to a final concentration of 1 U/µl.

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

SP6 RNA Polymerase is slightly more active at 40°C than at 37°C. Incubation at 40°C may be considered for RNA transcript containing strong secondary structures.

Quality Control Assays

Endonuclease Activity (Nicking): A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of supercoiled ϕX174 DNA and a minimum of 100 units of SP6 RNA Polymerase incubated for 4 hours at

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release): A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 100 units of SP6 RNA Polymerase incubated for 4 hours at 37ºC releases < 0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour): A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of SP6 RNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Promoter Specificity: A 50 µl reaction in RNAPol Reaction Buffer in the presence of 2 mM NTPs containing 1 µg of Lambda DNA as a template and a minimum of 100 units of SP6 RNA Polymerase incubated for 1 hour at 37°C results in < 1.5% of the amount of product incorporated as compared to a control reaction using SP6 DNA as a template.

Protein Purity Assay (SDS-PAGE): SP6 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion): A 10 µl reaction in RNAPol Reaction Buffer containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of SP6 RNA Polymerase is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Companion Products:

RNA Loading Dye (2X)
#B0363S 4 x 1 ml

RNase Inhibitor, Human Placenta
#M0307S 2,000 units
#M0307L 10,000 units

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

Pyrophosphatase, Inorganic (*E. coli*)
#M0361S 10 units
#M0361L 50 units

Low Range ssRNA Ladder
#N0364S 25 µg

ssRNA Ladder
#N0362S 25 µg

RNase Contamination Assay Kit
#E3320S 50 reactions

Vaccinia Capping System
#M2080S 400 units

mRNA Cap 2´-O-Methyltransferase
#M0366S 2,000 units

E. coli Poly(A) Polymerase
#M0276S 100 units
#M0276L 500 units

Ribonucleotide Solution Mix
#N0446S 10 µmol of each
#N0446L 50 µmol of each

Ribonucleotide Solution Set
#N0450S 10 µmol each
#N0450L 50 µmol each

T7 RNA Polymerase
#M0251S 5,000 units
#M0251L 25,000 units

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Promoter Specificity: A 50 µl reaction in RNAPol Reaction Buffer in the presence of 2 mM NTPs containing 1 µg of Lambda DNA as a template and a minimum of 100 units of SP6 RNA Polymerase incubated for 1 hour at 37°C results in < 1.5% of the amount of product incorporated as compared to a control reaction using SP6 DNA as a template.

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#E3320S 50 reactions

Vaccinia Capping System
#M2080S 400 units

mRNA Cap 2´-O-Methyltransferase
#M0366S 2,000 units

E. coli Poly(A) Polymerase
#M0276S 100 units
#M0276L 500 units

Ribonucleotide Solution Mix
#N0446S 10 µmol of each
#N0446L 50 µmol of each

Ribonucleotide Solution Set
#N0450S 10 µmol each
#N0450L 50 µmol each

T7 RNA Polymerase
#M0251S 5,000 units
#M0251L 25,000 units

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