**SP6 RNA Polymerase**

**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

**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.

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

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>AMOUNT</th>
<th>CONCENTRATION</th>
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<tbody>
<tr>
<td>H2O</td>
<td>X µl</td>
<td></td>
</tr>
<tr>
<td>10X Reaction Buffer</td>
<td>2 µl</td>
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</tr>
<tr>
<td>NTP</td>
<td>X µl</td>
<td>0.5 mM each</td>
</tr>
<tr>
<td>Template DNA</td>
<td>X µl</td>
<td>0.2–1 µg</td>
</tr>
<tr>
<td>RNase Inhibitor (optional)</td>
<td>0.5 µl</td>
<td>1 U/µl final</td>
</tr>
<tr>
<td>Fresh DTT (optional)</td>
<td>X µl</td>
<td>5 mM final</td>
</tr>
<tr>
<td>SP6 RNA Pol</td>
<td>2 µl</td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>20 µl</td>
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**Notes on use:**
- Incubate at 37°C for 1 hour. For shorter (< 300 nt) transcripts incubate at 37°C for 2–16 hours.
- 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 37°C.

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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 [³H] E. coli DNA and a minimum of 100 units of SP6 RNA Polymerase incubated for 4 hours at 37°C 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

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**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