SP6 RNA Polymerase

2 µl X µl 5 mM final
20 µl X µl 0.5 mM each
20 µl X µl 0.5 µl 1 U/µl final
AMOUNT X µl AMOUNT X µl 0.2–1 µg CONCENTRATION X µl X µl

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 LT22.

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

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 MgCl₂
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.

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>AMOUNT</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-100</td>
<td>X µl</td>
<td></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:
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.

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 40°C may be considered for RNA transcript containing strong secondary structures.

Incorporate at the closest temperature that is not less than the minimum specified temperature and may exceed the maximum specified temperature by up to 5°C.

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

Promoter Specificity: 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 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.

Promoter Specificity: 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 as determined by agarose gel electrophoresis.

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**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.

**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
- mRNA Cap 2’-O-Methyltransferase
  - #M0366S 2,000 units
- T7 RNA Polymerase
  - #N0450S 10 µmol each
  - #N0450L 50 µmol each
- Ribonucleotide Solution Set
  - #N0450S 10 µmol each
  - #N0450L 50 µmol each
- Sae II
  - #M0276S 100 units
  - #M0276L 500 units
- tRNA Loading Dye (2X)
  - #N0450S 10 µmol each
  - #N0450L 50 µmol each
- T7 RNA Polymerase
  - #M0251S 5,000 units
  - #M0251L 25,000 units
- RNase Contamination Assay Kit
  - #E3320S 50 reactions

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- T7 RNA Polymerase
  - #N0450S 10 µmol each
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- Ribonucleotide Solution Set
  - #N0450S 10 µmol each
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- Sae II
  - #M0276S 100 units
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- tRNA Loading Dye (2X)
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