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

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.

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>M.O.</td>
<td>X µl</td>
<td></td>
</tr>
<tr>
<td>10X Reaction Buffer</td>
<td>2 µl</td>
<td></td>
</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>
<td></td>
</tr>
</tbody>
</table>

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.

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 may be considered for RNA transcript containing strong secondary structures.

Source: An E. coli strain that carries the cloned gene for SP6 RNA Polymerase from Salmonella typhimurium LT2Z.
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 results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

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 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 #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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