SP6 RNA Polymerase

Product description

SP6 RNA Polymerase is a HIGHLY SPECIFIC DNA-dependent 5' →3' RNA polymerase that recognizes SP6 promoter sequences. SP6 RNA polymerase can catalyze the incorporation of NTP downstream of single- or double-stranded DNA SP6 promoters to synthesize RNA that is complementary to the template DNA downstream of the SP6 promoter. The SP6 promoter sequence is ATTTAGGTGACACTATAGAA, where the last G is the first base of transcription.

The RNA synthesized by SP6 RNA Polymerase can be used for hybridization probes, genomic DNA sequence analysis, RNase protection assays, antisense RNA synthesis, RNA templates for in vitro translation, substrates for RNA splicing studies, RNA secondary structure and RNA-protein interactions, nucleic acid amplification analysis, siRNA, miRNA and other small RNAs.

Composition and storage conditions

Components	2000 U	5000 U	10000 U
SP6 RNA Polymerase (20U/μl)	0.1 ml	0.25 ml	0.5 ml
10X SP6 Reaction Buffer	0.2 ml	0.5 ml	1 ml
Store the components at -20 °C.			

Experimental operation

1. Add the reaction system components (operating on ice) as shown in the table below:

Component	Volume
Template DNA	0.2~1 μg
10X SP6 Reaction Buffer	2 μl
NTP Mixture (10 mM each)	4 μl
Ribonuclease Inhibitor	20 U
SP6 RNA Polymerase	2 μl
DTT(optional)	5 mM final
Nuclease-free Water	to 20 μl

2. After setting up the reaction system as shown in the table above, mix gently (you can mix gently with a pipette or at the lowest speed with Vortex), and then centrifuge the pellet to retain the liquid.
3. Incubate at 37 °C for 1-2 h.
4. Add 2 μL of 0.5 M EDTA (pH 8.0) to the reaction system and mix well or cool at -20 °C to stop the reaction.
5. Electrophoresis analyzes transcription products or identifies transcription efficiency by other appropriate methods.

***Note:**

a. Transcription should be performed in the absence of RNases.

b. The reaction system needs to be configured at room temperature, and DNA can precipitate in the presence of spermidine at 4 °C.

c. The template DNA is linearized plasmid DNA containing the SP6 promoter, or a PCR product containing the SP6 promoter, or genomic DNA containing the SP6 promoter.

d. If the linearization of the template DNA is not complete, it will cause the transcription of RNA longer than expected, while reducing the proportion of transcripts of the expected length.

e. The above reaction system can be scaled up or reduced according to the actual situation.

The nature of the product

1. **Enzyme living units**

   At 37 °C, the amount of enzyme required to catalyze the incorporation of 1nmol AMP into the polynucleotide within 1 hour is defined as 1 active unit.

2. **Store the solution**

   50 mM Tris-HCl (pH 7.9 @ 25℃), 100 mM NaCl, 20 mM β-ME, 1 mM EDTA, 50% Glycerol, 0.1% (w/v) Triton® X-100

3. **10X SP6 Reaction Buffer**

   400 mM Tris-HCl(pH 7.9 @ 25°C), 60 mM $MgCl_2$, 10 mM DTT, 20 mM spermidine

4. **Heat deactivation conditions**

   Adding an appropriate amount of EDTA can inactivate sp6 RNA Polymerase. Chelating agents, sodium, potassium, or ammonium salts at concentrations greater than 150 mM can significantly inhibit the activity of SP6 RNA Polymerase.

Quality assurance

1. Protein Purity Test: SDS-PAGE Test Purity > 95%
2. Non-specific nuclease detection: At 37 °C reaction temperature, the 100U enzyme was incubated with Lambda DNA for 16 h, and the electrophoretic spectrum band did not change.
3. Promoter-specific detection: Incubate 100 U of enzyme with a reaction system containing 1 μg of Lamb DNA template and 2 mM NTP for 1 h at a reaction temperature of 37 °C, and the transcribed RNA <1% compared to a control reaction using SP6 DNA as a template.

4. Nucleic acid endonuclease activity detection: At 37 °C reaction temperature, after incubating 100 U enzyme with 1 μg of superhelix 174 DNA for 4 h, the plasmid DNA was detected using agarose gel electrophoresis, and the plasmid DNA was dissolved supercoiled < 10%.

5. Detection of exonuclease activity: 100 U enzymes were incubated with Hhind III- DNA at 37 °C for 4 h, the electrophoresis band did not change, and there was no non-specific exonuclease activity.

6. RNase detection: 20 U of enzyme and 200 ng of 300 base RNA substrate react at 37 °C for 4 h without change in the electrophoresis band of RNA.