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SP6 RNA Polymerase (Cloned)

Catalog Number AM2071

Pub. No. 4393893 **Rev.** B

Contents	Quantity	Storage conditions
SP6 RNA Polymerase (Cloned)	1000 Units (20 U/µL)	Store at –20°C. *Do not store in a frost-free freezer.*
10X Transcription Buffer: 400 mM Tris pH 7.8, 200 mM NaCl, 60 mM $MgCl_2$, 20 mM Spermidine HCl, 100 mM DTT	500 µL	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

Cloned SP6 RNA Polymerase is a high-purity SP6 RNA polymerase for synthesizing high specific activity RNA probes, biologically active mRNA, and antisense RNA.

Source: An *E. coli* strain harboring a plasmid that overexpresses SP6 RNA Polymerase.

Unit (U) definition: One unit is the amount of SP6 RNA polymerase required to catalyze the incorporation of 1 nmol of nucleoside triphosphate into acid-insoluble material in 60 minutes at 37°C.

Storage buffer (*not included*): 20 mM KPO_4, pH 7.7, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 50% glycerol (v/v), and detergent.

Using SP6 RNA Polymerase

SP6 RNA polymerase is highly specific for its own promoter, a conserved 23 bp sequence that is not efficiently recognized by T3 or T7 RNA polymerases. It will transcribe large amounts of RNA from DNA sequences (for example, plasmids, polymerase chain reaction (PCR) fragments, or hybridized oligonucleotides) downstream of its promoter, without cross-talk from nearby T3 or T7 promoters. Thus, RNA molecules transcribed from a linear template will be of a defined length. Using circular plasmid DNA as a template will result in heterogeneous transcripts of multiple lengths.

Labeled transcription reactions

The yield and amount of full-length RNA transcript obtained depend on the ratio of template DNA to the concentration of the limiting ribonucleoside triphosphate (rNTP) in the transcription reaction. Typically, three nucleotides are present at 500 µM and the nucleotide used for labeling is at various concentrations, depending on the desired specific activity of the probe (Butler and Chamberlin, 1982; Krieg and Melton, 1984). The limiting nucleotide should generally be present at a minimum of 3 µM to maximize synthesis of full-length RNA transcripts. Under standard assay conditions, >50% of the label is incorporated in 30 minutes into RNA.

We find that temperature is not a critical variable, although 37°C is frequently recommended as the appropriate incubation temperature. In fact, lower temperatures seem to favor the synthesis of full-length transcripts under conditions of limiting nucleotide concentration. It may be convenient to run these reactions at room temperature.

The following reaction conditions will yield labeled RNA probe suitable for use with Northern blots containing a moderately abundant mRNA.

Typical labeled transcription reaction conditions (20-µL reactions):

- 1 µg template DNA
- 2 µL 10X Transcription Buffer (included)
- 500 µM (final) rNTPs (A, G, C)
- 50 µCi [α-^{32}P]UTP (800 mCi/mmol, 10 mCi/mL)
- 0.1–1.0 U/µL (final) RNase Inhibitor (Cat. no. AM2682, AM2684)
- 40 U SP6 RNA Polymerase
- Nuclease-free water to a final volume of 20 µL

Incubate 30 minutes at 37°C.

Treat the reaction with DNase I to remove DNA template (optional; see below) or simply stop the transcription reaction by adding 2 µL of 0.2 M EDTA and/or heating to 65°C.

Unlabeled transcription reactions

In this reaction, rNTP levels are not limiting, and large amounts of RNA are synthesized throughout the incubation period. Frequently, more than 4 µg RNA may be synthesized per µg of input DNA.

Note: This protocol may be altered to include nonisotopically-labeled rNTPs. See **Technical Bulletin 173, *Methods for Nonisotopic Labeling*** for a protocol and sources of these nucleotides.

Typical unlabeled transcription reaction conditions (20-µL reaction):

- 1 µg template DNA
- 2 µL 10X Transcription Buffer (included)
- 500 µM (final) rNTPs (A, C, G, U)
- 0.1–1.0 U/µL (final) RNase Inhibitor (Cat. no. AM2682, AM2684)
- 40 U SP6 RNA Polymerase
- Nuclease-free water to a final volume of 20 µL

Incubate 60 minutes at 37°C.

Treat the reaction with DNase I to remove DNA template (optional; see below) or simply stop the transcription reaction by adding 2 µL of 0.2 M EDTA and/or heating to 65°C.

(Optional) Removal of DNA template

Remove the DNA template by digestion with 2 units of DNase I (RNase-free; Cat. no. AM2222, AM2224) or TURBO™ DNase (Cat. no. AM2238, AM2239) for 15 minutes at 37°C. Inactivate the DNase by adding 2 µL 0.2 M EDTA and heating at 70°C for 10 minutes, or by phenol/chloroform extraction.

For information about other post-reaction options, such as removal of unincorporated nucleotides, see the user guide for the MAXIscript® Kit (Cat. no. AM1312), available at **www.lifetechnologies.com**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

References

Butler, E.T. and Chamberlin, M.J. (1982) *J Biol Chemistry* 257, 5772–5778.

Krieg, P.A. and Melton, D.A. (1984) *Nucl Acids Res* 12, 7057–7070.