Thermo SCIENTIFIC

PRODUCT INFORMATION

T7 RNA Polymerase

#EP0111 5000 u

Lot: **Expiry Date:**

Concentration: 20 u/µl
Supplied with: 1.25 ml of 5X Transcription Buffer

Store at -20°C

In total 2 vials. BSA included

www.thermoscientific.com/fermentas

Description

Bacteriophage T7 RNA Polymerase is a DNAdependent RNA polymerase with strict specificity for its respective double-stranded promoter. The enzyme catalyzes the 5'→3' synthesis of RNA on either single-stranded DNA or double-stranded DNA downstream from the promoter. T7 RNA Polymerase accepts modified nucleotides (e.g., biotin-, digoxigenin-, fluorescein-labeled nucleotides) as substrates for RNA synthesis.

Applications

Synthesis of unlabeled and labeled RNA that can be used:

- for hybridization (1), *in vitro* RNA translation (2);
- as aRNA (3), siRNA (4), substrate in RNase protection assays (5), template for genomic DNA sequencing (6);
- in studies of RNA secondary structure and RNA-protein interactions (7), RNA splicing (8).

Source

E.coli cells with a cloned gene encoding this enzyme.

Molecular Weight

99 kDa monomer.

Rev.6 53

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction (adsorbed on DE-81) in 60 minutes at 37°C.
Enzyme activity is assayed in the following mixture: 40 mM Tris-HCl (pH 8.0), 6 mM $MgCl_2$, 10 mM DTT, 2 mM spermidine, 0.5 mM of each NTP, 0.6 MBq/ml [^{3}H]-ATP, 20 µg/ml plasmid DNA containing the T7 promoter sequence.

Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM DTT, 0.1 mg/ml BSA, 0.03% (v/v) ELUGENT Detergent and 50% (v/v) glycerol.

5X Transcription Buffer

200 mM Tris-HCl (pH 7.9 at 25°C), 30 mM $MgCl_2$, 50 mM DTT, 50 mM NaCl and 10 mM spermidine.

Inhibition and Inactivation

- Inhibitors: metal chelators, enzyme activity is reduced by 50% at NaCl or KCl concentration above 150 mM.
- Inactivated by heating at 70°C for 10 min or by addition of EDTA.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 200 units of T7 RNA Polymerase with 1 µg of pUC19 DNA for 1 hour at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 200 units of T7 RNA Polymerase with 1 µg of [^{3}H]-RNA for 1 hour at 37°C.

Functional Assay

T7 RNA Polymerase was tested in *in vitro* transcription reaction.

Quality authorized by: Jurgita Zilinskiene

(continued on back page)

Protocol for *in vitro* transcription

1. Linearize template DNA with a restriction enzyme. Extract DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in DEPC-treated Water (#R0601).
2. Prepare the following reaction mixture:

5X Transcription buffer	10 µl
ATP/GTP/CTP/UTP Mix, 10 mM each	10 µl (2 mM final concentration)
Linear template DNA	1 µg
Thermo Scientific RiboLock RNase Inhibitor (#EO0381)	1.25 µl (50 u)
T7 RNA Polymerase	30 u
DEPC-treated water (#R0601)	to 50 µl

3. Incubate at 37°C for 2 hours.
4. Optional: To remove template DNA add 2 µl (2 u) of DNase I, RNase-free (#EN0521), mix and incubate at 37°C for 15 min.
5. Inactivate DNase I by phenol/chloroform extraction.

Note

- The transcription reaction should be performed under conditions that exclude contamination with RNases. The tips, tubes and water should be nuclease free. All the solutions should be made up in nuclease free water. Wearing gloves is advisable.
- The reaction mixture should be prepared at room temperature, since DNA may precipitate in the presence of spermidine at 4°C.
- Under the conditions described above, more than 10 µg RNA per 1 µg template DNA is obtained.
- The yield of proper length transcripts decreases if the template DNA is incompletely linearized due to a read-through reaction and accumulation of longer transcripts of a variable length.
- The reaction mixture can be scaled up or down.

Protocol for synthesis of radiolabeled RNA probes of high specific activity

1. Linearize template DNA with a restriction enzyme. Extract DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in DEPC-treated Water (#R0601).
2. Prepare the following reaction mixture:

5X Transcription buffer	4 µl
3 NTP mix, 10 mM each	1 µl (0.5 mM final concentration)
100 µM CTP (#R0451)	2.4 µl (12 µM final concentration)
[α-^{32}P]-CTP, ~30 TBq/mmol (800 Ci/mmol)	1.85 MBq (50 µCi)
Linear template DNA	0.2-1.0 µg
RiboLock™ RNase Inhibitor (#EO0381)	0.4 µl (20 u)
T7 RNA Polymerase	20 u
DEPC-treated water (#R0601)	to 20 µl

3. Incubate at 37°C for 2 hours.
4. Stop the reaction by cooling at -20°C.
5. Determine the percentage of label incorporated into RNA.

Note

- RNA synthesized under the conditions described above usually has a specific activity of 3-5 x10^8dpm/µg.
- RNA can be radiolabeled with [^{32}P], [^{35}S] or [^{3}H]-ribonucleotides. The use of 1.85 MBq (50 µCi) of 5'-[α-^{32}P]-CTP, ~30 TBq/mmol (800 Ci/mmol), 11.1 MBq (300µCi) of 5'-[α-^{35}S]-UTP, >37 TBq/mmol (>1000 Ci/mmol), 0.925 MBq (25 µCi) of 5,6-[^{3}H]-UTP, 1.1-2.2 TBq/mmol (30-60 Ci/mmol) for 20 µl reaction mixture is recommended.
- The yield of full-length transcripts is reduced when the final concentration of labeled NTP is below 12 µM.

References

1. Melton, D.A., et al., Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter, Nucleic Acids Res.,12, 7035-7056, 1984.
2. Krieg, P.A., Melton, D.A., Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs, Nucleic Acids Res., 12, 7057-7070, 1984.
3. Melton, D.A., Injected antisense RNAs specifically block messenger RNA translation *in vivo*, Proc. Natl. Acad. Sci. USA, 82, 144-148, 1985.
4. Bernstein, E., et al., Role for bidentate ribonuclease in the initiation step of RNA interference, Nature, 409, 363-366, 2001.
5. Peebles, C.L., et al., A self-splicing RNA excises an intron lariat, Cell, 44, 213-223, 1986.
6. Church, G.M., Gilbert, W., Genomic sequencing, Proc. Natl. Acad. Sci. USA, 81, 1991-1995, 1984.
7. Witherell, G.W., et al., Cooperative binding of R17 coat protein to RNA, Biochemistry, 29, 11051-11057, 1990.
8. Krainer, A.R., et al., Normal and mutant human β-globin pre-mRNAs are faithfully and efficiently spliced *in vitro*, Cell, 36, 993-1005, 1984.
9. Jorgensen, E.D.,et al., Specific contacts between the bacteriophage T3, T7, and SP6 RNA polymerases and their promoters, J. Biol. Chem., 266, 645-651, 1991.