



## PRODUCT INFORMATION

# T3 RNA Polymerase

Pub. No. MAN0012019

Rev. Date 16 September 2016 (Rev. B.00)

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Lot: \_                      Expiry Date: \_

Store at -20 °C

Components	#EP0101	#EP0103
T3 RNA Polymerase	20 U/μL 2000 U	200 U/μL HC, 10000 U
5X Transcription Buffer	0.8 mL	2 × 1.25 mL

BSA included

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## Description

Bacteriophage T3 RNA Polymerase is a DNA-dependent 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. T3 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 (see protocols on back page):

- 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 the T3 RNA Polymerase.

## Molecular Weight

99 kDa monomer.

### **Definition of Activity Unit**

One unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction in 60 minutes at 37 °C.

### **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<sub>2</sub>, 50 mM DTT, 50 mM NaCl and 10 mM spermidine.

### **Inhibition and Inactivation**

- Inhibitors: metal chelators, enzyme activity is reduced above 250 mM by 50% at NaCl or KCl concentration.
- Inactivated by heating at 70 °C for 10 min or by addition of EDTA.

## **CERTIFICATE OF ANALYSIS**

### **Endodeoxyribonuclease Assay**

No detectable degradation was observed after incubation of supercoiled plasmid DNA with T3 RNA Polymerase.

### **Ribonuclease Assay**

No detectable degradation was observed after incubation of [3H]-RNA with T3 RNA Polymerase.

### **Endo-, exodeoxyribonuclease and phosphatase Assay**

No detectable degradation after incubation of single stranded and double stranded radiolabeled oligonucleotides with T3 RNA Polymerase.

### **Functional Assay**

T3 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:

<b>5X Transcription buffer</b>	10 $\mu$ L
<b>ATP/GTP/CTP/UTP Mix, 10 mM each</b>	10 $\mu$ L (2 mM final concentration)
<b>Linear template DNA</b>	1 $\mu$ g
<b>Thermo Scientific RiboLock RNase Inhibitor (#EO0381)</b>	1.25 $\mu$ L (50 U)
<b>T3 RNA Polymerase</b>	30 U
<b>DEPC-treated water (#R0601)</b>	to 50 $\mu$ L

3. Incubate at 37 °C for 2 hours.
4. Optional: To remove template DNA add 2  $\mu$ L (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  $\mu$ g RNA per 1  $\mu$ g 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:

<b>5X Transcription buffer</b>	4 $\mu$ L
<b>3 NTP mix, 10 mM each</b>	1 $\mu$ L (0.5 mM final concentration)
<b>100 <math>\mu</math>M CTP (#R0451)</b>	2.4 $\mu$ L (12 $\mu$ M final concentration)
<b>[<math>\alpha</math>-<sup>32</sup>P]-CTP, ~30 TBq/mmol (800 Ci/mmol)</b>	1.85 MBq (50 $\mu$ Ci)
<b>Linear template DNA</b>	0.2-1.0 $\mu$ g
<b>RiboLock™ RNase Inhibitor (#EO0381)</b>	0.4 $\mu$ L (20 U)
<b>T3 RNA Polymerase</b>	20 U
<b>DEPC-treated water (#R0601)</b>	to 20 $\mu$ L

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<sup>8</sup>dpm/ $\mu$ g.
- RNA can be radiolabeled with [<sup>32</sup>P], [<sup>35</sup>S] or [<sup>3</sup>H]-ribonucleotides. The use of 1.85 MBq (50  $\mu$ Ci) of 5'-[ $\alpha$ -<sup>32</sup>P]-CTP, ~30 TBq/mmol (800 Ci/mmol), 11.1 MBq (300 $\mu$ Ci) of 5'-[ $\alpha$ -<sup>35</sup>S]-UTP, >37 TBq/mmol (>1000 Ci/mmol), 0.925 MBq (25  $\mu$ Ci) of 5,6-[<sup>3</sup>H]-UTP, 1.1-2.2 TBq/mmol (30-60 Ci/mmol) for 20  $\mu$ L reaction mixture is recommended.
- The yield of full-length transcripts is reduced when the final concentration of labeled NTP is below 12  $\mu$ M.

## References

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8. Krainer, A.R., et al., Normal and mutant human  $\beta$ -globin pre-mRNAs are faithfully and efficiently spliced *in vitro*, *Cell*, 36, 993-1005, 1984.
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