For life science research only.
Not for use in diagnostic procedures.

Roche

T7 RNA Polymerase

From *Escherichia coli* BL 21/pAR 1219
Nucleoside-triphosphate: RNA nucleotidyltransferase (DNA-directed), EC 2.7.7.6

Cat. No. 10 881 767 001 1,000 U

Cat. No. 10 881 775 001 5,000 U

Version 22
Content version: March 2016

Store at −15 to −25° C

Product Overview

Pack Content

Vial	Content
T7 RNA Polymerase	• 1,000 U • 5,000 U Enzyme storage buffer: 10 mM Potassium phosphate, 200 mM KCl, 0.1 mM EDTA, 30 mM 2-mercaptoethanol, 50% glycerol (v/v), 0.1% Tween 20, pH 7.9 (+4°C).
Supplied Transcription buffer	Buffer composition (10x conc.): 0.4 M Tris-HCl, pH 8.0 (+20°C), 60 mM $MgCl_2$, 100 mM dithiothreitol, 20 mM spermidine.

Storage and Stability
Stable at -15 to -25°C until the expiration date printed on the label.

Product Description
T7 RNA polymerase is commonly used to transcribe DNA which has been cloned into vectors which have two phage promoters in opposite orientation. RNA can be selectively synthesized from either strand of the insert DNA with different polymerases.

Homogeneously labeled single-stranded RNA can be generated with this system. Transcripts can be non-radioactively labeled with biotin or DIG-11-UTP* or radioactively labeled to high specific activity with [α-^{32} P] or [α-^{35}S] labeled nucleotides.

Volume Activity
≥20 U/µl

One unit is the enzyme activity which incorporates 1 nmol CMP in acid-precipitable RNA products within 60 min at +37°C (1).

Enzyme Characteristics
The enzyme comprises a single polypeptide chain of 98 kDa (2). The T7 RNA polymerase requires a DNA template and Mg^{2+} as cofactor for the synthesis of RNA. The enzyme is strongly stimulated by BSA or spermidine. In contrast to bacterial RNA polymerases, T7 RNA polymerase is not inhibited by the antibiotic rifampicin.

Promotor Specifity
T7 RNA polymerase is extremely promoter-specific and only transcribes bacteriophage T7 DNA or DNA cloned downstream of a T7 promoter.

Although the T7 and T3 promoter sequences differ only by 3 bp, T7 RNA polymerase only transcribes DNA cloned downstream of its promoter (3).

Applications

- RNA or DNA blotting techniques (4, 5),
- *in situ* hybridization (6),
- microarray target synthesis (16, 17)
- genomic sequencing (7).
- For RNA sequencing, cloned DNA is transcribed with the enzyme in the presence of 3´-dNTPs (8, 9). These nucleotides act as chain terminators in a similar manner to the ddNTP's in the Sanger sequencing method (10).
- RNase protection studies (11).
  Transcripts synthesized by the enzyme are used as precursor RNA for studies on RNA splicing and processing (12).
- It is also possible to synthesize capped RNA *in vitro* with addition of m7GpppG or m7GpppA in excess over GTP or ATP during the transcription reaction (13, 14). The generated antisense RNA can be introduced into cells to suppress the expression of the corresponding genes (15).

Standard Transcription Assay

Additional Reagents Required

- lin. template DNA including T7 RNA promotor
- Ribonucleoside triphosphates
- labeled nucleotide (according to application)
- RNAse inhibitor
- Water, PCR Grade

Radioactive Assay
Pipet the following components into a microfuge tube, mix and make up to a final volume of 20 µl:

Reagent	Volume/Concentration
Template DNA	0.5 µg
Nucleotides ATP, GTP, CTP, UTP	each 0.5 mM final
Labeled nucleotide [α-^{32}P] CTP [400 Ci/mmol; 15 TBq/mmol]	0.1 µl aqueous solution
Supplied buffer, 10 x conc.	2 µl
T7 RNA Polymerase	20 U
RNase Inhibitor	20 U
Water, PCR Grade	x µl

Incubation time: 20 min at +37°C

Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or heat to +65°C.

Non-Radioactive Assay
Pipet the following components into a microfuge tube, mix and make up to a final volume of 20 µl:

Reagent	Volume/Concentration
Template DNA	1 µg
Nucleotides ATP, GTP, CTP	each 1 mM final
Labeled nucleotide: UTP, and DIG-11-UTP (or Biotin-16-UTP)	0.65 mM final 0.35 mM final
Supplied buffer, 10 x conc.	2 µl
T7 RNA Polymerase	40 U
RNase Inhibitor	20 U
Water, PCR Grade	x µl

Incubation time: 2 hours at +37°

Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or heat to +65°C.

Cold Assay
Pipet the following into a microfuge tube, mix and make up to a final volume of 20 µl:

Reagent	Volume/Concentration
Template DNA	1 µg
Nucleotides ATP, GTP, CTP, UTP	each 1 mM final
Supplied buffer, 10 x conc.	2 µl
T7 RNA Polymerase	40 U
RNase Inhibitor	20 U
Water, PCR Grade	x µl

Incubation time: 2 hours at +37°C

Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or heat to +65°C.

DNA Removal
DNA can be removed with 20 U DNase I, RNase-free and incubation for 15 min at +37°C.

Control of Transcript
Transcripts can be checked for length and integrity by native or denaturing gel electrophoresis.

Purification of Transcript
Radioactively labeled transcripts can be purified from non-incorporated ribonucleoside triphosphates by column chromatography (*e.g.,* Quick Spin Columns Sephadex G-50 fine, Cat. No. 11 274 015 001) or from enzymatic components by phenol extraction.

Do not phenol/chloroform extract your DIG-labeled probe because it will partition into the organic phase.

Determination of Labeling Efficiency
The yield of the labeling reaction can be determined by trichloracetic acid precipitation and for nonradioactive labeling please refer to our DIG Application Manual for Filter Hybridization.

Quality control

Test Buffer
40 mM Tris-HCl, pH 8.0 (+20°C), 6 mM $MgCl_2$, 10 mM dithiothreitol, 2 mM spermidine, pH approximately 8.0 (+20°C).

Absence of Endonucleases
1. 1 µg λDNA is incubated with T7 RNA polymerase for 4 hours at +37°C in 25 µl test buffer. The number of enzyme units which show no degradation of λDNA is > 100 U.
2. 1 µg *Eco* RI/*Hind* III fragments of λDNA is incubated with T7 RNA polymerase for 4 hours at +37°C in 25 µl test buffer. The number of enzyme units which show no alteration of the banding pattern is > 100 U.

Absence of Nicking Activity
1 µg pBR322 DNA is incubated with T7 RNA polymerase for 4 hours at +37°C in 25 µl test buffer. The number of enzyme units which show no relaxing of supercoiled structure is > 100 U.

Absence of RNases
4 µg MS2 RNA are incubated with T7 RNA polymerase for 4 hours at +37°C in 50 µl test buffer. The number of enzyme units which show no degradation of MS2 RNA is > 100 U.

Performance in Transcription Assay
T7 RNA polymerase is function tested in the SP6/T7 Transcription Kit (Cat. No. 10 999 644 001). The standard assay with 1.0 µg of the corresponding template contained in the control DNA labeled with 6 mM DIG-11-UTP mix gives sensitivity by direct spot-test ≤ 1 pg incorporation in 20 min.

References

1 Davanloo, P. *et al.* (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2035.
2 Stahl, S. & Zinn, K. (1981) *J. Mol. Biol.* **148,** 481.
3 Brown, J. E. *et al.* (1986) *Nucleic Acids Res.* **14**, 3521.
4 Southern, E. M. (1975) *J. Mol.* **98,** 503.
5 Smith, G. E. & Summers, M. D. (1980) *Anal. Biochem.* **109,** 123.
6 Zinn, K. *et al.* (1983) *Cell* **34**, 865.
7 Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci.USA* **81**, 1981.
8 Klement, J. F. *et al.* (1986) *Gene Anal. Techn.* **3**, 59.
9 Parin, J. D. *et al.* (1986) *DNA* **5**, 167.
10 Sanger, F. *et al.* (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463.
11 Pables, C. L. *et al.* (1986) *Cell* **44**, 213.
12 Krainer, A. *et al.* (1984) *Cell* **36**, 993.
13 Contreras, R. *et al.* (1982) *Nucleic Acids Res.* **10**, 6353.
14 Konarska, M. & Sharp, P. (1989) *Cell Vol.* **57**, No. 3, 423–431.
15 Melton, D. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 144.
16 Lu et al (1990) *Gene* **86**:185-192
17 Lockhart *et al.* (1996) *Nature Biotechnol.* **14**:1675-1680

Ordering Information.

Product	Pack size	Cat. No.
SP6 RNA Polymerase	1,000 U 5,000 U	10 810 274 001 11 487 671 001
T3 RNA Polymerase	1,000 U 5,000 U	11 031 163 001 11 031 171 001
DIG RNA Labeling Kit (SP6/T7)	1 kit (2 x 10 labeling reactions)	11 175 025 910
DIG RNA Labeling Mix	40 µl (20 reactions)	11 277 073 910
SP6/T7 Transcription Kit	1 kit (2 x 20 reactions)	10 999 644 001
DNase I, rec.RNase-free	10,000 U	04 716 728 001
Protector RNase Inhibitor	2,000 U 10,000 U	03 335 399 001 03 335 402 001
UTP	40 µmol (400 µl)	11 140 949 001
DIG-11-UTP	250 nmol (25 µl)	11 209 256 910
Biotin-16-UTP	250 nmol (25 µl)	11 388 908 910
Fluorescein-12-UTP	250 nmol (25 µl)	11 427 857 910
Ribonucleoside Triphosphate Set	1 set 4 x 20 µmol	11 277 057 001
Water, PCR Grade	100 ml (4 x 25 ml) 25 ml (25 x 1 ml) 25 ml (1 x 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001

Changes to Previous Version

Update of Quality Control Test
Editorial Changes

Trademarks

All product names and trademarks are the property of their respective owners

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Disclaimer of License

For patent license limitations for individual products please refer to: List of biochemical reagent products

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany