Certificate of Analysis

T3 RNA Polymerase:

Part No.	Size (units)
P208C	1,000
P402A	(High Conc.) 2,500

Part# 9PIP208

Revised 1/18

Description: SP6, T3 and T7 RNA Polymerases are DNA-dependent RNA polymerases that exhibit extremely high specificity for their cognate promoter sequences. For example, only T3 DNA or DNA cloned downstream from an T3 promoter can serve as a template for T3 RNA Polymerase-directed RNA synthesis (1,2); T3 RNA Polymerase does not recognize SP6 or T7 RNA Polymerase promoter sequences as a start site for transcription. SP6, T3 and T7 RNA Polymerases will incorporate ^{32}P, ^{35}S and ^{3}H nucleotide phosphates.T3 RNA Polymerase is available in the Riboprobe® Systems.

Applications of phage RNA polymerases include:

- Synthesis of RNA transcripts for hybridization probes (3).
- Synthesis of large amounts of nonlabeled RNA (3).
- In vitro synthesis of capped RNA transcripts (3).
- RNase protection assays.

Transcription Optimized 5X Buffer (Cat.# P1181): When the Transcription Optimized 5X Buffer supplied with this enzyme is diluted 1:5, it has a composition of 40mM Tris (pH 7.9), 6mM $MgCl_2$, 2mM spermidine and 10mM NaCl.

100mM DTT, (Cat.# P1171): Add to a final concentration of 10mM in a standard transcription reaction.

Enzyme Storage Buffer: T3 RNA Polymerase is supplied in 20mM potassium phosphate buffer (pH 7.7), 1mM EDTA, 10mM DTT, 0.1M NaCl, 0.1% Triton® X-100 and 50% (v/v) glycerol.

Source: *E. coli* strain expressing a recombinant clone.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 5nmol of rCTP into acid-insoluble product in 1 hour at 37°C in a total volume of 100µl (4). The reaction conditions are: 40mM Tris-HCl (pH 7.9), 10mM NaCl, 6mM $MgCl_2$, 10mM DTT, 2mM spermidine, 0.05% Tween®-20, 0.5mM each of rATP, rGTP, rCTP and rUTP, 0.5µCi [^{3}H]rCTP and 2µg of supercoiled pSP6/T3 vector DNA. See the unit concentration on the Product Information Label.

Usage Note: Please refer to Reference 2 to for additional information and applications for T3 RNA Polymerase.

Storage Temperature: Store at –20°C. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information Label.

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Quality Control Assays

Activity Assays

RNA Synthesis Assay: T3 RNA Polymerase is tested for RNA synthesis using the same conditions as for Unit Definition (above) except that unlabeled rCTP is limited to 12µM, the Tween®-20 is excluded and pGEM® Express Positive Control DNA (Cat.# P2561) is used as template. Separate reactions are performed using 1, 2, 5, 10 and 20 units of enzyme for 1 hour at 37°C. Minimum passing specification is ≥65% incorporation of [^{3}H]rCTP using 20 units of enzyme.

Transcription Assay: T3 RNA Polymerase is tested in a transcription assay using pGEM® Express Positive Control DNA incubated for 1 hour at 37°C with 5 or 10 units of enzyme. Transcripts are denatured by heating at 65°C for 10 minutes in formamide/formaldehyde buffer and resolved in a 1% agarose gel in TAE buffer. Specification is to obtain intact transcripts of the correct size with no degradation.

Contaminant Activity

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or RNA is incubated with 100 units of T3 RNA Polymerase in Transcription Optimized 1X Buffer for 1 hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting the TCA-soluble material. Minimum passing specification is ≤1% release for DNase and RNase activity.

Physical Purity: Purity is >90% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

References

1. Butler, E.T. and Chamberlain, M.J. (1982) *J. Biol. Chem.* **257**, 5772–8.
2. Melton, D.A. *et al.* (1984) *Nucl. Acids Res.* **12**, 7035–56.
3. *Riboprobe® in vitro Transcription Systems Technical Manual* #TM016, Promega Corporation.
4. Knoche, K., Stevens, J. and Bandziulis, R. (1997) *Promega Notes* **61**, 2–5.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

I. Standard Applications

Protocols for three standard applications of phage RNA polymerases are given. Reference 3 contains additional information and applications for the phage RNA polymerases. Please read the pertinent section(s) and prepare any reagents as appropriate. Gloves should be worn when working with transcription reagents or RNA transcripts to prevent RNase contamination.

Materials to Be Supplied by the User

All materials except α-^{32}P and the DNA template, linearized, can be found in Sections II (Composition of Buffers and Solutions) and Section III (Related Products).

- DNA template, linearized
- Nuclease-Free Water
- Recombinant RNasin® Ribonuclease Inhibitor
- rNTP mix, or rNTP capping mix
- [α-^{32}P]rCTP (400Ci/mmol, 10Ci/ml)
- Ribo m^{7}G Cap Analog, 5mM (Cat.# P1711)

A. Synthesis of High Specific Activity RNA Probes

1. In a microcentrifuge tube, add the following reagents at room temperature in the order listed:

Transcription Optimized 5X Buffer	4µl
DTT, 100mM	2µl
Recombinant RNasin® Ribonuclease Inhibitor	20 units
rATP, rGTP and rUTP mix, 2.5mM each	4µl
rCTP, 100µM	2.4µl
DNA template, linearized (in water or TE buffer at 0.2–1.0µg/µl)	1µl
[α-^{32}P]rCTP (50µCi at 10mCi/ml)	5µl
Phage RNA polymerase	20 units
Nuclease-Free Water to final volume of	20µl

2. Incubate for 1 hour at 37°C.

B. Synthesis of Nonlabeled RNA

1. In a microcentrifuge tube, add the following reagents at room temperature in the order listed:

T3 transcription 5X buffer	20µl
DTT, 100mM	10µl
Recombinant RNasin® Ribonuclease Inhibitor	100 units
rNTP mix (see Section II)	20µl
DNA template, linearized (in water or TE buffer at 2–5µg)	2µl
Phage RNA polymerase	40 units
Nuclease-Free Water to final volume of	100µl

2. Incubate for 2 hours at 37°C.

C. Synthesis in vitro of Capped RNA Transcripts

1. In a microcentrifuge tube, add the following reagents at room temperature in the order listed:

Transcription Optimized 5X Buffer	10µl
DTT, 100mM	5µl
Recombinant RNasin® Ribonuclease Inhibitor	50 units
rNTP capping mix (see Section II)	5µl
Ribo m^{7}G Cap Analog, 5mM	5µl
DNA template, linearized (in water or TE buffer at 1µg/µl)	5µl
Phage RNA polymerase	40 units
Nuclease-Free Water to final volume of	50µl

2. Incubate for 1 hour at 37°C. To increase the yield of RNA, add an additional 40 units of phage RNA polymerase and incubate for 1 hour.

II. Composition of Buffers and Solutions

rNTP mix

2.5mM rCTP
2.5mM rATP
2.5mM rGTP
2.5mM rUTP
in Nuclease-Free Water

rNTP capping mix

5mM rATP
5mM rUTP
5mM rCTP
0.5mM rGTP
in Nuclease-Free Water

Transcription Optimized 5X Buffer (provided)

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

III. Related Products

A. Related Systems

Product	Cat.#
Riboprobe® System—SP6	P1420
Riboprobe® System—T3	P1430
Riboprobe® System—T7	P1440
Riboprobe® System Buffers	P1121
RiboMAX™ Large Scale RNA Production System—SP6	P1280
RiboMAX™ Large Scale RNA Production System—T7	P1300
TNT® T7 Quick Coupled Transcription/Translation System	L1170
TNT® T7 Quick Coupled Transcription/Translation System, Trial Size	L1171
TNT® SP6 Quick Coupled Transcription/Translation System	L2080
TNT® SP6 Quick Coupled Transcription/Translation System, Trial Size	L2081
TNT® SP6 Coupled Reticulocyte Lysate System	L4600
TNT® T3 Coupled Reticulocyte Lysate System	L4950
TNT® T7 Coupled Reticulocyte Lysate System	L4610
TNT® T7/SP6 Coupled Reticulocyte Lysate System	L5020
TNT® T7/T3 Coupled Reticulocyte Lysate System	L5010
TNT® SP6 Coupled Reticulocyte Lysate System, Trial Size	L4601
TNT® T7 Coupled Reticulocyte Lysate System, Trial Size	L4611

B. Related Products

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
pGEM® Express Positive Control Template	10µg (2 × 5µg)	P2561
rATP, 100mM	400µl	E6011
rUTP, 100mM	400µl	E6021
rGTP, 100mM	400µl	E6031
rCTP, 100mM	400µl	E6041
rATP, rCTP, rGTP and rUTP, each at 100mM	400µl each	E6000
Nuclease-Free Water	50ml (2 × 25ml)	P1193
Ribo m^{7}G Cap Analog	10 A_{254} units	P1711
	25 A_{254} units	P1712

Product	Concentration	Size	Cat.#
Recombinant RNasin®	20–40u/µl	2,500u	N2511
Ribonuclease Inhibitor	20–40u/µl	10,000u	N2515

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