

Product Contents

T3 RNA Polymerase:

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

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 ³²P, ³⁵S and ³H nucleotide phosphates.

SP6, T3 and T7 RNA Polymerases are available in Promega's RiboMAX™(a,b,c) and Riboprobe®(a) 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 MgCl₂, 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 MgCl₂, 10mM DTT, 2mM spermidine, 0.05% Tween®-20, 0.5mM each of rATP, rGTP, rCTP and rUTP, 0.5µCi [³H]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.

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^(e) (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 [³H]rCTP using 20 units of enzyme.

Transcription Assay: T3 RNA Polymerase is tested in a transcription assay using pGEM® Express Positive Control DNA^(e) 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) Bacteriophage SP6— specific RNA polymerase I isolation and characterization of the enzyme. *J. Biol. Chem.* **257**, 5772.
2. Melton, D.A. *et al.* (1984) Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucl. Acids Res.* **12**, 7035.
3. *Riboprobe® in vitro Transcription Systems Technical Manual #TM016*, Promega Corporation.
4. Knoche, K., Stevens, J. and Bandziulis, R. (1997) A comparative study of T7 RNA polymerase quality. *Promega Notes* **61**, 2.

(b)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(c)The RiboMAX™ Large Scale RNA Production Systems—T7 and T3 (Cat.# P1290 and P1300) are covered by U.S. Pat. No. 5,256,555 and are sold under a license from Ambion, Inc. They are intended for research use only. Parties wishing to use these products for other applications should contact Ambion, Inc.

(d)U.S. Pat. No. 5,283,179 and other patents.

(e)U.S. Pat. No. 4,766,072.

(f)U.S. Pat. Nos. 5,492,817, 5,665,563 and other patents.

Part# 9PIP208

Revised 12/04



Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

© 1997–2004 Promega Corporation. All Rights Reserved.

(e)U.S. Pat. No. 5,552,302 and other patents. *Inhibitors of Angiogenin*, which comprises a segment of human PRI, is the subject of U.S. Pat. No. 4,966,964 and other patents assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.

pGEM, RNasin, Riboprobe and TNT are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office. RiboMAX is a trademark of Promega Corporation.

Coomassie is a registered trademark of Imperial Chemical Industries. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc. Tween is a registered trademark of ICI Americas, Inc.

All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Part# 9PIP208
Printed in USA. Revised 12/04

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 -³²P and the DNA template, linearized, can be found in Sections II and III.

(Solution compositions are provided in Section II.)

- DNA template, linearized
- Nuclease-Free Water
- Recombinant RNasin® Ribonuclease Inhibitor^(a)
- rNTP mix, or rNTP capping mix
- [α -³²P]rCTP (400Ci/mmol, 10Ci/ml)
- Ribo m7G 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	4 μ l
DTT, 100mM	2 μ l
Recombinant RNasin® Ribonuclease Inhibitor ^(a)	20 units
rATP, rGTP and rUTP mix, 2.5mM each	4 μ l
rCTP, 100 μ M	2.4 μ l
DNA template, linearized (in water or TE buffer at 0.2–1.0 μ g/ μ l)	1 μ l
[α - ³² P]rCTP (50 μ Ci at 10mCi/ml)	5 μ l
Phage RNA Polymerase	20 units
Nuclease-Free Water to final volume of	20 μ l

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	20 μ l
DTT, 100mM	10 μ l
Recombinant RNasin® Ribonuclease Inhibitor ^(a)	100 units
rNTP mix (see Section II)	20 μ l
DNA template, linearized (in water or TE buffer at 2–5 μ g)	2 μ l
Phage RNA Polymerase	40 units
Nuclease-Free Water to final volume of	100 μ l

2. Incubate for 2 hour 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	10 μ l
DTT, 100mM	5 μ l
Recombinant RNasin® Ribonuclease Inhibitor ^(a)	50 units
rNTP Mix (see Section II)	5 μ l
Ribo m7G Cap Analog, 5mM	5 μ l
DNA template, linearized (in water or TE buffer at 1 μ g/ μ l)	5 μ l
Phage RNA Polymerase	40 units
Nuclease-Free Water to final volume of	50 μ l

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	rATP
2.5mM	rGTP
2.5mM	rUTP
2.5mM	rCTP

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(a)	P1420
Riboprobe® System—T3(a)	P1430
Riboprobe® System—T7(a)	P1440
Riboprobe® System Buffers	P1121
RiboMAX™ Large Scale RNA Production System—SP6(a,b)	P1280
RiboMAX™ Large Scale RNA Production System—T3(a,b,c)	P1290
RiboMAX™ Large Scale RNA Production System—T7(a,b,c)	P1300
TNT® T7 Quick Coupled Transcription/Translation System(a,b,d,f)	L1170
TNT® T7 Quick Coupled Transcription/Translation System, Trial Size(a,b,d,f)	L1171
TNT® SP6 Quick Coupled Transcription/Translation System(a,b,d,f)	L2080
TNT® SP6 Quick Coupled Transcription/Translation System, Trial size(a,b,d,f)	L2081
TNT® SP6 Coupled Reticulocyte Translation System(a,b,d,f)	L4600
TNT® T3 Coupled Reticulocyte Translation System(a,b,d,f)	L4950
TNT® T7 Coupled Reticulocyte Translation System(a,b,d,f)	L4610
TNT® T7/SP6 Coupled Reticulocyte Translation System(a,b,d,f)	L5020
TNT® T7/T3 Coupled Reticulocyte Translation System(a,b,d,f)	L5010
TNT® SP6 Coupled Reticulocyte Translation System, Trial Size(a,b,d,f)	L4601
TNT® T7 Coupled Reticulocyte Translation System, Trial Size(a,b,d,f)	L4611

B. Related Products

Product	Size	Cat.#
SP6 Promoter Primer	2 μ g	Q5011
T3 Promoter Primer	2 μ g	Q5741
T7 Promoter Primer	2 μ g	Q5021
pGEM® Express Positive Control Template	10 μ g (2 × 5 μ g)	P2561
rATP, 100mM	400 μ l	E6011
rUTP, 100mM	400 μ l	E6021
rGTP, 100mM	400 μ l	E6031
rCTP, 100mM	400 μ l	E6041
rATP, rCTP, rGTP and rUTP, each at 100mM	400 μ l each	E6000
Nuclease-Free Water	50ml (2 × 25ml)	P1193
Ribo m7G Cap Analog	10 A ₂₅₄ units	P1711
	25 A ₂₅₄ units	P1712

Product	Conc.	Size	Cat.#
Recombinant RNasin®	20–40u/ μ l	2,500u	N2511
Ribonuclease Inhibitor ^(a)	20–40u/ μ l	10,000u	N2515