Certificate of Analysis

DNA Polymerase I Large (Klenow) Fragment:

Part No.	Size (units)
M220A	150
M220C	500

Part# 9PIM220

Revised 8/13

Enzyme Storage Buffer: Klenow Fragment is supplied in 50mM Tris-HCl (pH 7.5), 1mM DTT, 0.1mM EDTA and 50% (v/v) glycerol.

Klenow 10X Buffer (M195A): The Klenow 10X Buffer supplied with this enzyme has a composition of 500mM Tris-HCl (pH 7.2), 100mM $MgSO_4$ and 1mM DTT.

Source: Recombinant strain of *E. coli* (1).

Storage Conditions: See the Product Information Label for storage recommendations. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10nmol of deoxynucleotide into acid-precipitable material in 30 minutes at 37°C. The reaction conditions are: 67mM potassium phosphate (pH 7.5), 6.7mM $MgCl_2$, 1mM DTT, 50µg/ml activated calf thymus DNA, and 33µM each of dCTP, dATP, dGTP and [^{3}H]dTTP. See the unit concentration on the Product Information Label.

Quality Control Assays

Activity Assay

Unit Activity Assay: See unit definition.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Form I (supercoiled) plasmid DNA is incubated with 10 units of Klenow Fragment in Klenow 1X Buffer for 5 hours at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Physical Purity: The purity is >80% as judged by SDS-polyacrylamide gels with Coomassie® blue staining. Klenow Fragment is free from intact DNA polymerase and small fragment as indicated by SDS-polyacrylamide gel electrophoresis.

Reference

1. Joyce, C.M. and Grindley, N.D. (1983) Construction of a plasmid that overproduces the large proteolytic fragment (Klenow fragment) of DNA polymerase I of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **80**, 1830–4.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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

Part# 9PIM220

Printed in USA. Revised 8/13

Promega

Usage Information

I. Description

DNA Polymerase I Large (Klenow) Fragment consists of a single polypeptide chain (68kDa) that lacks the 5´→3´ exonuclease activity of intact *E. coli* DNA polymerase I but retains its 5´→3´ polymerase, 3´→5´ exonuclease and strand displacement activities. The 5´→3´ polymerase activity of Klenow Fragment can be used in the following applications: a) to fill in 5´-protruding ends with unlabeled or labeled dNTPs (1); b) to sequence single- or double-stranded DNA templates (2); c) for in vitro mutagenesis experiments using synthetic oligonucleotides (3); d) for cDNA second strand synthesis (4); e) to generate single-stranded DNA probes (5). The 3´→5´ exonuclease activity can be used to generate blunt ends from a 3´-overhang (6). Klenow Fragment, Exonuclease Minus, which is deficient in both the 5´→3´ and the 3´→5´ exonuclease activities, is primarily used for sequencing of DNA templates. For a detailed review of Klenow and other DNA polymerases, see reference 7.

II. Reaction Conditions for Klenow Applications

Many of the applications in which Klenow Fragment is used require restriction digestion of the DNA. We recommend purifying the DNA by using the Wizard® DNA Clean-Up System (Cat.# A7280) or phenol:chloroform extraction and ethanol precipitation before filling 5´-protruding ends using Klenow Fragment. For optimal activity, use the Klenow 10X Buffer supplied with the enzyme. Klenow Fragment is also active in many restriction enzyme buffers, and some users may choose to perform the fill-in reaction directly in the restriction buffer. For other applications, such as sequencing, the optimal reaction conditions are described in the individual protocols below.

A. Filling 5´-Protruding Ends with Unlabeled dNTP

Digest 1–4µg of DNA (in a volume of 20µl) with an appropriate restriction enzyme that will generate a 5´-overhang. The optimal reaction conditions for filling are: 50mM Tris-HCl (pH 7.2), 10mM $MgSO_4$, 0.1mM DTT, 40µM of each dNTP, 20µg/ml acetylated BSA and 1 unit of Klenow Fragment per microgram of DNA. Incubate the reaction at room temperature for 10 minutes. Stop the reaction by heating the mixture for 10 minutes at 75°C.

Note: Klenow Fragment, Exonuclease Minus, will leave a single-base 3´-overhang for a significant proportion of the DNA fragments during the fill-in reaction (8). Therefore, these fragments should not be used in blunt end cloning experiments.

B. Filling 5´ Protruding Ends With Labeled dNTP

Digest 1–4µg of DNA (in a volume of 20µl) with an appropriate restriction enzyme that will generate a 5´-overhang. Add 20µCi of the desired [α-^{32}P]dNTP (400–800Ci/mmol), 1µl of an appropriate 5mM dNTP solution and 1–4 units of Klenow Fragment to the reaction mixture. Incubate the reaction for 15 minutes at 30°C. Stop the reaction by adding 1µl of 0.5M EDTA (20mM final concentration) to the mixture, or by heating the mixture for 10 minutes at 75°C. This method is particularly suitable for labeling restriction fragments to use as size standards, since all fragments are labeled equally and will have the same intensity on an autoradiogram (6).

C. Generation of Single-Stranded DNA Probes

Denature the DNA template by heating it in a microcentrifuge tube for 10 minutes at 95–100°C. Rapidly chill the tube in an ice bath. To generate a single-stranded DNA probe, prepare the following reaction mixture: 500ng/ml denatured DNA template (25ng optimum), 50mM Tris-HCl (pH 8.0), 5mM $MgCl_2$, 2mM DTT, 0.2M HEPES (pH 6.6), 150µg/ml random hexadeoxyribonucleotides, 400µg/ml BSA, 20µM of each unlabeled dNTP, 333nM [α-^{32}P]dNTP (3,000Ci/mmol) and 5 units of Klenow Fragment. Incubate the reaction for 60 minutes at room temperature. Stop the reaction by adding 1µl of 0.5M EDTA (20mM final concentration) to the mixture or by heating the mixture for 10 minutes at 75°C.

D. Dideoxy Sequencing

Note: We recommend the use of Klenow Fragment, Exonuclease Minus, for this protocol.

The protocol detailed here is suitable for sequencing both single-stranded and denatured double-stranded DNA templates. If the template is single-stranded, proceed directly to the sequencing protocol. If the template is double-stranded, follow the denaturation procedure outlined below.

Denaturation protocol: Pipet 0.8–4µg of supercoiled plasmid into a microcentrifuge tube and add sterile deionized water to a final volume of 18µl. Add 2µl of 2M NaOH/2mM EDTA and mix the solutions by pipeting. Incubate the DNA for 15 minutes at 37°C. Add 2µl of 2M ammonium acetate (pH 4.6), and vortex to mix. Add 112µl of 95% ethanol, vortex briefly and incubate the tube for 15 minutes at –70°C. Centrifuge the tube for 15 minutes at 12,000 x *g* in a microcentrifuge. Carefully remove the supernatant and wash the pellet with 500µl of cold (–20°C) 70% ethanol. Centrifuge the tube for 5 minutes at 12,000 x *g* in a microcentrifuge. Carefully remove **all** of the supernatant and resuspend the DNA in 5µl of deionized water. Proceed to the sequencing protocol.

Sequencing protocol: In a 0.5ml microcentrifuge tube, combine 0.8–4µg of single-stranded denatured DNA template, 0.8–2pmol of primer (maintain a 1:1 molar ratio of template:primer), 1.5µl of sequencing reaction 10X buffer [70mM Tris-HCl (pH 7.5), 70mM $MgCl_2$, 300mM NaCl, 100mM DTT, 1mM EDTA (pH 8.0)] and sterile, redistilled water to a final volume of 10µl. To anneal the primer to the template DNA, incubate the mixture for 10 minutes at 55°C and then slowly cool it to room temperature. Add 2µl of [α-^{35}S]dATP (1,000Ci/mmol) and 2 units of Klenow Fragment, Exonuclease Minus. Mix the components by pipeting. Centrifuge the tube at 12,000 x *g* for 10 seconds in a microcentrifuge to collect the mixture in the bottom of the tube. Transfer 2.5µl of the reaction mixture to each of 4 tubes marked G, A, T and C. Add 2µl of the appropriate d/ddNTP mix (see Table 1) to each tube, mix by pipeting and centrifuge briefly in a microcentrifuge. Incubate the reaction at room temperature for 20 minutes. Add 2µl of chase solution (125µM of each dNTP) and incubate the reaction for an additional 20 minutes at room temperature. Add 4µl of stop solution (95% formamide, 0.5% xylene cyanol, 0.5% bromophenol blue, 10mM NaOH) to each of the reactions. Heat the reactions for 3 minutes at 95°C just prior to loading them onto a sequencing gel.

Table 1. Composition of dNTP/ddNTP Mixtures.

Nucleotide	G Mixture	A Mixture	T Mixture	C Mixture
7-deaza-dGTP	5µM	100µM	100µM	100µM
dTTP	100µM	100µM	5µM	100µM
dCTP	100µM	100µM	100µM	10µM
ddGTP	120µM	–	–	–
ddATP	–	100µM	–	–
ddTTP	–	–	500µM	–
ddCTP	–	–	–	100µM

III. Additional Information

Molecular Weight: Klenow Fragment is a 68kDa monomer (7).
Heat Inactivation: Klenow Fragment may be inactivated by incubation for 10 minutes at 75°C.
Inhibitors: Klenow Fragment is inhibited by the adenosine analog adenosine 2´,2´-riboepoxide 5´-triphosphate (9).

IV. References

1. Anderson, S. *et al.* (1980) A short primer for sequencing DNA cloned in the single-stranded phage vector M13mp2. *Nucl. Acids Res.* **8**, 1731–43.
2. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463–7.
3. Wallace, R.B. *et al.* (1980) Directed deletion of a yeast transfer RNA intervening sequence. *Science* **209**, 1396–400.
4. Houdebine, L.M. (1976) Synthesis of DNA complementary to the mRNAs for milk proteins by *E. coli* DNA polymerase I. *Nucl. Acids Res.* **3**, 615–30.
5. Feinberg, A.P. and Vogelstein, B. (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**, 6–13.
6. Tabor, S. and Struhl, K. (1987) In: *Current Protocols in Molecular Biology*, Ausubel, F.M. *et al.*, eds., John Wiley and Sons, New York, NY.
7. *Methods in Molecular Biology*, Vol. 16 (1993) Burrell, M.M., ed., Humana Press, Toronto, Canada.
8. Clark, J.M., Joyce, C.M. and Beardsley, G.P. (1987) Novel blunt-end addition reactions catalyzed by DNA polymerase I of *Escherichia coli*. *J. Mol. Biol.* **198**, 123–7.
9. Abboud, M.M. *et al.* (1987) Apparent suicidal inactivation of DNA polymerase by adenosine 2´,3´-riboepoxide 5´-triphosphate. *J. Biol. Chem.* **253**, 3415–21.

Part# 9PIM220
Printed in USA. Revised 8/13

Certificate of Analysis

Part# 9PIM205

Revised 9/13

DNAPolymerase I:

Part No.	Size (units)
M205A	500
M205B	2,500

DNA Polymerase I 10X Reaction Buffer (M195A): The DNA Polymerase 10X Reaction Buffer supplied with this enzyme has a composition of 500mM Tris-HCl (pH 7.2 at 25°C), 100mM $MgSO_4$ and 1mM DTT.

Enzyme Storage Buffer: DNA Polymerase I is supplied in 50mM Tris-HCl (pH 7.5 at 25°C), 1mM DTT, 0.1mM EDTA and 50% (v/v) glycerol.

Source: Purified from an *E. coli* strain expressing a recombinant clone (1).

Storage Temperature: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10nmol of deoxyribonucleotides into trichloroacetic acid insoluble form in 30 minutes at 37°C in 67mM potassium phosphate (pH 7.4), 6.7mM $MgCl_2$, 1mM DTT, 50µg/ml activated calf thymus DNA and 33µM each dNTP. See the unit concentration on the Product Information Label.

Quality Control Assays

Activity Assay

Unit Activity Assay: See unit definition above.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated in 25 units of DNA Polymerase I for 5 hours at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

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

Reference

1. Kelley, W.S., Chalmers, K. and Murray, N.E. (1977) Isolation and characterization of a lambdapolA transducing phage. *Proc. Natl. Acad. Sci. USA* **74**, 5632–6.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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.

Part# 9PIM205
Printed in USA. Revised 9/13

Usage Information

I. Description

DNA Polymerase I catalyzes template-directed polymerization of nucleotides into duplex DNA in a 5´→3´ direction. DNA Polymerase I also possesses a 3´→5´ exonuclease activity or "proofreading" function, which lowers the error rate during DNA replication, and contains a 5´→3´ exonuclease activity, which enables the enzyme to replace nucleotides in the growing strand of DNA by nick translation (1). DNA Polymerase I is capable of catalyzing *de novo* synthesis of synthetic homopolymers and provides a convenient method for the preparation of a variety of defined DNA substrates in the laboratory (2).

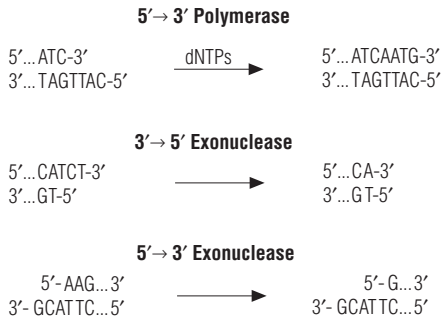


Figure 1. Properties of DNA Polymerase I.

II. Reaction Conditions

A. Filling-In of 5´-Overhang

This procedure has been adapted from reference 3.

Mix the following in a microcentrifuge tube: 8.5µl water, 5µl DNA (1–5µg digested DNA containing 5´-overhangs), 1.5µl 1M Tris-HCl (pH 7.5), 2.5µl 0.1M $MgCl_2$, 2.5µl 0.1M β-mercaptoethanol, 5µl 1mM dNTP mixture (2µl each of 10mM dATP, dCTP, dGTP, dTTP and 12µl water) and 0.5µl DNA Polymerase I (2.5 units). Incubate at 12°C for 1 hour. Heat at 68°C for 10 minutes to inactivate the enzyme.

B. Blunt-Ending of 3´-Overhang

This procedure has been adapted from reference 3.

The 3´-overhang is first removed by the exonuclease activity of DNA Polymerase I. Because removal of more nucleotides may occur (generating a 5´-protruding end), repair is performed after addition of dNTPs in order to generate a blunt end. In a microcentrifuge tube, prepare a 1mM mixture of the four dNTPs (2µl each of 10mM dATP, dCTP, dGTP, dTTP and 12µl water). In another microcentrifuge tube, mix 8.5µl water, 5µl DNA(containing 1–5µg digested DNA with 3´-overhangs), 1.5µl 1M Tris-HCl (pH 7.5), 2.5µl 0.1M $MgCl_2$, 2.5µl 0.1M β-mercaptoethanol, 0.5µl DNA Polymerase I (2.5 units). Incubate at 12°C for 10 minutes. Add 5µl of the 1mM dNTP mixture to the DNA. Incubate at 12°C for 1 hour. Heat at 68°C for 10 minutes to inactivate the enzyme.

C. Nick Translation

This reaction may be scaled between 10-100µl volume, but the components should be kept in the same proportions as in the standard reaction. Set up the following reaction in a microcentrifuge tube: 18µl water, 10µl nucleotide mix (prepared by mixing equal volumes of the 3 unlabeled 300µM nucleotides chosen minus the nucleotide selected as label), 5µl nick translation 10X buffer (see Section IV), 5µl sample DNA (at 0.2µg/µl), 7µl [α-^{32}P]dCTP (400Ci/mmol at 10mCi/ml) and 5µl DNA Polymerase I/DNase I mix (see Section IV). Incubate at 15°C for 1 hour. Add 5µl stop solution (0.25M EDTA [pH 8.0]).

III. Miscellaneous Information

Source: DNA Polymerase I is purified from the recombinant *E. coli* strain CM5199 (4), which is a lysogen carrying a lambda pol A transducing phage (5).
Molecular Weight: 109,000 Daltons.
Activator: Mg^{2+}.
Inactivator: 68°C for 10 minutes (3).

IV. Composition of Buffers and Solutions

Nick translation 10X buffer

500mM	Tris-HCl (pH 7.2)
100mM	$MgSO_4$
1.0mM	DTT

DNA Polymerase I/DNase I mix

50%	glycerol
50mM	Tris-HCl (pH 7.2)
10mM	$MgSO_4$
0.1mM	DTT
0.5mg/ml	nuclease-free BSA
1,000u/ml	DNA Polymerase I
3u/ml	RQ1 RNase-Free DNase (DNase I) (Cat.# M6101)

Prepare the buffer solution and then add the DNA Polymerase I and RQ1 RNase-Free DNase to a final concentration of 1,000u/ml and 3u/ml, respectively.

V. References

1. Kelly, R.B. *et al.* (1970) Enzymatic synthesis of deoxyribonucleic acid. XXXII. Replication of duplex deoxyribonucleic acid by polymerase at a single strand break. *J. Biol. Chem.* **245**, 39–45.
2. Harwood, S.J. *et al.* (1970) *Micrococcus luteus* deoxyribonucleic acid polymerase. Studies of the enzymic reaction and properties of the deoxyribonucleic acid product. *J. Biol. Chem.* **245**, 5614–24.
3. Perbal, B. (1988) *A Practical Guide to Molecular Cloning, 2nd ed.*, John Wiley and Sons.
4. Kelley, W.S. and Stump, K.H. (1979) A rapid procedure for isolation of large quantities of *Escherichia coli* DNA polymerase I utilizing a lambdapolA transducing phage. *J. Biol. Chem.* **254**, 3206–10.
5. Kelley, W.S., Chalmers, K. and Murray, N.E. (1977) Isolation and characterization of a lambdapolA transducing phage. *Proc. Natl. Acad. Sci. USA* **74**, 5632–6.

Part# 9PIM205
Printed in USA. Revised 9/13

Taq DNA Polymerase

Cat. Nos.
18038-018 (100 units) **18038-042 (500 units)**
18038-067 (1500 units) **18038-240 (5000 units)**

Conc: 5 U/µl **Store at –20°C (non-frost-free)**

Licensed for PCR

Description

Taq DNA Polymerase is isolated from *Thermus aquaticus* YT1. The enzyme consists of a single polypeptide with a molecular weight of approximately 94 kDa. *Taq* DNA polymerase is heat-stable and will synthesize DNA at elevated temperatures from single-stranded templates in the presence of a primer.

	Kit Size			
Component	**100 U**	**500 U**	**1500 U**	**5,000 U**
Taq DNA Polymerase	20 µl	100 µl	300 µl	1000 µl
10X PCR Buffer, Minus Mg	1.25 ml	2.5 ml	7.5 ml	20 ml
50 mM Magnesium Chloride	1 ml	1 ml	3 ml	10 ml

Storage Buffer

20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, Stabilizers

10X PCR Buffer

200 mM Tris-HCl (pH 8.4), 500 mM KCl

The PCR Buffer is supplied as a 10X concentrate and should be diluted for use.

Unit Definition

One unit incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C. Unit assay conditions: 25 mM TAPS (pH 9.3), 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.5 mg/ml activated salmon sperm DNA, 0.2 mM dATP, dCTP, dGTP, dTTP

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of *Taq* DNA Polymerase, primers, $MgCl_2$, and template DNA) vary and need to be optimized.

Critical parameters and troubleshooting information are documented in reference 1. PCR reactions should be assembled in a DNA-free environment. Use of "clean" dedicated automatic pipettors and aerosol resistant barrier tips are recommended. **Always** keep the control DNA and other templates to be amplified isolated from the other components.

1. Add the following components to a sterile 0.5-ml microcentrifuge tube sitting on ice:

Components	**Volume**	**Final Conc.**
10X PCR buffer minus Mg	10 µl	1X
10 mM dNTP mixture	2 µl	0.2 mM each
50 mM $MgCl_2$	3 µl	1.5 mM
Primer mix (10 µM each)	5 µl	0.5 µM each
Template DNA	1–20 µl	n/a
Taq DNA Polymerase (5 U/µl)	0.2–0.5 µl	1.0–2.5 units
Autoclaved distilled water	to 100 µl	n/a

   We recommend preparing a master mix for multiple reactions, to minimize reagent loss and enable accurate pipetting.
2. Mix contents of tube and overlay with 50 µl of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.

Basic PCR Protocol, continued

4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. Perform 25–35 cycles of PCR amplification as follows:
   Denature 94°C for 45 s
   Anneal 55°C for 30 s
   Extend 72°C for 1 min 30 s
6. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at –20°C until use.
7. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

"Hot-start" Protocol

In the "hot-start" method, the addition of *Taq* DNA Polymerase is withheld until the reaction temperature is at 80°C, to ensure high specificity of the products being synthesized.

1. Add all components as in the Basic PCR Protocol, except for the *Taq* DNA Polymerase.
2. Mix contents of tube and overlay with 50 µl of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.
4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. After denaturation at 94°C, maintain the reaction at 80°C.
6. Add 0.2–0.5 µl of *Taq* DNA Polymerase (1.0–2.5 U) to each reaction. Be certain to add the enzyme beneath the layer of oil.
7. Continue with 25–35 cycles of denaturation, annealing and extension as in the Basic PCR Protocol.

Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Reference

1. Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J., eds. (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, CA.

Limited Use Label License No. 1: Thermostable Polymerases

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the U.S.: 5,789,224, 5,618,711, 6,127,155.The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Platinum™ *Pfx* DNA Polymerase

Catalog Numbers 11708-013, 11708-021, and 11708-039
Doc. Part No. 11708.pps **Pub. No.** MAN0000996 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ Platinum™ *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from *Thermococcus* species *KOD*. The Platinum™ *Pfx* DNA Polymerase possesses proofreading 3′ to 5′ exonuclease activity and is a highly processive enzyme with fast chain extension capability.

The Platinum™ *Pfx* DNA Polymerase is provided in inactive form, due to specific binding of the Platinum™ antibody. Polymerase activity is restored after a PCR denaturation step at 94°C, providing an automatic "hot start" for increased specificity, sensitivity, and yield. Platinum™ *Pfx* DNA Polymerase is ideal for demanding PCR applications such as site-directed mutagenesis and PCR expression cloning.

For problematic and/or GC–rich templates, PCR_x Enhancer Solution is included with each kit. See "Guidelines for PCRx Enhancer Solution" on page 2.

Contents and storage

Contents	Cat. No.			Storage
	11708-013 (100 rxns)	11708-021 (250 rxns)	11708-039 (500 rxns)	
Platinum™ *Pfx* DNA Polymerase (2.5 U/µL)	100 U	250 U	500 U	–20°C
50 mM Magnesium sulfate	1 mL	1 mL	1 mL	
10X *Pfx* Amplification Buffer	1 mL	2 x 1 mL	3 x 1 mL	
10X PCR_x Enhancer Solution	1 mL	2 x 1 mL	3 x 1 mL	

Note: Unit (U) definition: One unit of Platinum™ *Pfx* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Procedural guidelines

- Platinum™ *Pfx* DNA Polymerase will not work in reactions that contain dUTP in the dNTP mix or the primers.
- Use a 1X final concentration of 10X *Pfx* Amplification Buffer as a general starting point. A higher final buffer concentration (1.5–2X) may enhance reaction yield and specificity.
- We recommend using 1 unit of Platinum™ *Pfx* for most targets. For targets above 2 kb, up to 1.25 units may be used. Note that more enzyme may be inhibitory.
- The recommended starting concentration of $MgSO_4$ is 1 mM.
- Use an annealing temperature of 55–60°C as a starting point.
- Only use PCR_x Enhancer Solution for problematic and/or GC–rich templates.

Guidelines for PCR_x Enhancer Solution

PCR_x Enhancer Solution is included as an optional component for problematic and/or GC-rich templates. Use PCR_x Enhancer Solution in combination with 10X *Pfx* Amplification Buffer, *not* as a substitute.

PCR_x Enhancer Solution lowers the DNA melting temperature (T_m), reducing the maximum primer annealing temperature approximately 2°C per 1X PCR_x Enhancer Solution concentration, while at the same time expanding the effective annealing temperature over a much broader range. To determine optimal concentrations and conditions, start with an annealing temperature of 55–60°C and vary the amount of 10X PCR_x Enhancer Solution. For targets with higher GC content (60–90%), test 10X PCR_x Enhancer Solution at final concentrations of 0.5X, 1X, 2X, and 3X.

Perform the PCR

The following protocol is specific for Platinum™ *Pfx* DNA Polymerase. For optimal performance, carefully follow these instructions.

1. Add the following components to a sterile microcentrifuge tube at room temperature or on ice.

Component	Volume for one 50-µL reaction	Final concentration
10X *Pfx* Amplification Buffer	5–10 µL[1]	1–2X
10 mM dNTP mixture	1.5 µL	0.3 mM each
50 mM $MgSO_4$	1 µL	1 mM
Primer mix (10 µM each)	1.5 µL	0.3 µM each
Template DNA (10 pg to 200 ng)	≥1 µL	as required
Platinum™ *Pfx* DNA Polymerase	0.4 µL	1 U
Autoclaved, distilled water	to 50 µL	—

[1] Use 5 µL (1X final concentration) of buffer as a general starting point.

Note: The volumes provided are for a single reaction. Prepare a master mix of common components for multiple reactions.

2. Mix the tube contents. Cap the tube, then centrifuge briefly to collect the contents.
3. Denature the template for 2–5 minutes at 94°C. Note that a longer denaturation time (up to 5 minutes) may increase yield and specificity.
4. Perform 25–35 cycles of PCR amplification as follows:

Step	Three-step cycling		Two-step cycling	
	Temp.	Time	Temp.	Time
Denature	94°C	15 seconds	94°C	15 seconds
Anneal	55°C	30 seconds	—	—
Extend	68°C	1 minute per kb	68°C	1 minute per kb

Note: Two-step cycling can be used for long primers with high T_m.

5. Maintain the reaction at 4°C after cycling. Samples can be stored at –20°C until use.
6. Analyze the products by agarose gel electrophoresis.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0000996

Revision	Date	Description
A.0	5 May 2016	Format, style, and legal updates
—	11 May 2010	Baseline for this revision history

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

Certificate of Analysis

AMV Reverse Transcriptase:

Part No.	Size (units)
M510A	300
M510F	1,000
M900A	(High Conc.) 600

Part# 9PIM510

Revised 9/11

AMV Reverse Transcriptase 5X Reaction Buffer (M515A): The AMV Reverse Transcriptase 5X Reaction Buffer supplied with this enzyme has a composition of 250mM Tris-HCl (pH 8.3 @ 25°C), 250mM KCl, 50mM $MgCl_2$, 2.5mM spermidine and 50mM DTT.

Enzyme Storage Buffer: AMV Reverse Transcriptase (AMV-RT) is supplied in 200mM potassium phosphate (pH 7.2 @ 4°C), 0.2% Triton® X-100, 2mM DTT and 50% glycerol.

Source: Purified from avian myeloblastosis virus particles.

Storage Conditions: Store at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C. The reaction conditions are: 50mM Tris-HCl (pH 8.3), 40mM KCl, 8.75mM $MgCl_2$, 10mM DTT, 0.1mg/ml acetylated BSA, 1mM radiolabeled dTTP and 0.25mM poly(A):oligo(dT). See the unit concentration on the Product Information Label.

Usage Notes:

1. The AMV Reverse Transcriptase 5X Reaction Buffer is intended for use in standard first-strand cDNA synthesis reactions. No deoxynucleotides are in the buffer; therefore, this buffer must not be substituted for the Promega RiboClone® AMV RT First-Strand 5X Buffer (Part# C121A), a component of the Universal RiboClone® cDNA Synthesis System (Cat.# C4360), which does have dNTPs. The Access RT-PCR System (Cat.# A1250) utilizes AMV Reverse Transcriptase and *Tfl* DNA Polymerase to provide a combined reverse transcription and PCR without intermediate handling. The reaction buffer provided in the Access RT-PCR System is not the same as the 5X Reaction Buffer provided with AMV-RT. The two buffers are not interchangeable.
2. The formulation of AMV Reverse Transcriptase 5X Reaction Buffer is **not** compatible with M-MLV Reverse Transcriptase.
3. Up to 10µl of an RT reaction containing AMV-RT and the supplied AMV Reverse Transcriptase Reaction Buffer can be added to PCR amplification reactions that use *Taq* DNA Polymerase. If GoTaq® DNA Polymerase (Cat.# M3001) or PCR Master Mix (Cat.# M7501) are used, up to 25µl of the RT reaction can be added to a 50µl PCR.

Quality Control Assays

Activity Assay

First-Strand cDNA Synthesis: First-strand cDNA, of a 1.2kb Control RNA (from Cat.# C4360), is synthesized using 30 units of AMV Reverse Transcriptase per microgram of template, an oligo(dT) primer and a radiolabeled dNTP. The minimum specification is the conversion of >12% of mRNA to cDNA. Full-length cDNA must be observed by gel electrophoresis and autoradiography.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 25 units of AMV Reverse Transcriptase in 50mM Tris (pH 8.3), 40mM KCl, 7mM $MgCl_2$, 10mM DTT for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 25 units of AMV Reverse Transcriptase in 4mM Tris (pH 8.3), 3.2mM KCl, 0.56mM $MgCl_2$, 0.8mM DTT for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is <1% release for DNase and <3% release for RNase.

Physical Purity: AMV Reverse Transcriptase is a 170kDa heterodimer with an α-subunit of 65kDa and a β-subunit of 94kDa. The purity is >80% in 2 bands (2 subunits) as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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

Part# 9PIM510

Printed in USA. Revised 9/11

Usage Information

1. Description

AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrids (1). It requires a primer (DNA primers are more efficient than RNA primers) as well as Mg^{2+} or Mn^{2+}. The enzyme possesses an intrinsic RNase H activity. Please refer to the **Usage Notes**, which appear on the other side of this document, before using this enzyme.

Applications of AMV RT include:

- First-strand synthesis of cDNA from RNA molecules (2).
- Sequencing of RNA transcripts (3).

2. Standard Applications

A. First-Strand Synthesis of cDNA

Reagents to Be Supplied by the User

- 10mM dNTP mix (Cat.# U1511, U1515 or prepared from 100mM dNTP sets Cat.# U1240, U1330, U1410, U1420; see Section 3.)
- Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511)
- sodium pyrophosphate, 40mM (prewarmed to 42°C)
- Oligo(dT) (Cat.# C1101) or Random Primers (Cat.# C1181)
- Nuclease-Free Water (Cat.# P1193)
- EDTA (50mM)
- [α-^{32}P]dCTP (>400Ci/mmol, 10mCi/ml)

1. The following procedure (4) uses **2µg** of RNA. In a sterile, nuclease-free microcentrifuge tube, add the primer to the RNA sample. Use 0.5µg primer/µg RNA in a total volume of ≤11µl in water. **Do not** alter the ratio of primer to template RNA. Heat to 70°C for 5 minutes. Chill the tube on ice for 5 minutes and centrifuge briefly to collect the solution at the bottom of the tube.
2. Add the following components to the annealed primer/template in the order shown.

AMV Reverse Transcriptase 5X Reaction Buffer	5µl
dNTP mix	2.5µl
RNasin® Ribonuclease Inhibitor	40 units
sodium pyrophosphate, 40mM (prewarmed to 42°C)	2.5µl
AMV RT	30 units
Nuclease-Free Water to final volume	25µl

3. Mix gently by flicking the tube and transfer 5µl of the reaction mixture to another tube containing 2–5µCi [α-^{32}P]dCTP. Do not add label to the remaining 20µl reaction. **Note:** We recommend using [α-^{32}P]dCTP that is less than 1 week old.
4. Incubate for 60 minutes at 42°C for oligo(dT) primers or at 37°C for random hexamer primers.
5. Place the reactions, labeled and unlabeled, on ice and add 95µl of 50mM EDTA to the labeled (tracer) reaction. The reaction volume should now total 100µl. The tracer reaction may be used for an incorporation assay and gel analysis (4).
6. Perform second-strand synthesis using the unlabeled first-strand reaction (see references 4 and 5). No phenol extraction or ethanol precipitation is necessary.

B. Sequencing of RNA Transcripts

A protocol for sequencing RNA transcripts may be found in reference 3.

3. Composition of Buffers and Solutions

dNTP mix

10mM each dATP, dCTP, dGTP and dTTP in water. (Prepare from 100mM stock solutions)

4. References

1. Kacian, D.L. (1977) Methods for assaying reverse transcriptase. *Meth. Virol.* **6**, 143.
2. Krug, M.S. and Berger, S.L. (1987) First-strand cDNA synthesis primed with oligo(dT). *Meth. Enzymol.* **152**, 316–25.
3. Mierendorf, R.C. and Pfeffer, D. (1987) Sequencing of RNA transcripts synthesized in vitro from plasmids containing bacteriophage promoters. *Meth. Enzymol.* **152**, 563–6.
4. *Universal RiboClone® cDNA Synthesis System Technical Manual* #TM038, Promega Corporation.
5. Sambrook, J. Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64.

Part# 9PIM510
Printed in USA. Revised 9/11

Certificate of Analysis

M-MLV Reverse Transcriptase:

Part No.	Size (units)
M170A	10,000
M170B	50,000

Part# 9PIM170

Revised 2/11

Enzyme Storage Buffer: M-MLV Reverse Transcriptase is supplied in 20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% Nonidet® P-40 and 50% glycerol.

M-MLV Reverse Transcriptase 5X Reaction Buffer (M531A): When the M-MLV Reverse Transcriptase 5X Reaction Buffer supplied with this enzyme is diluted 1:5, it has a composition of 50mM Tris-HCl (pH 8.3 @ 25°C), 75mM KCl, 3mM $MgCl_2$ and 10mM DTT.

Source: Purified from an *E. coli* strain expressing a recombinant clone (1).

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

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C. The reaction conditions are: 50mM Tris-HCl (pH 8.3), 7mM $MgCl_2$, 40mM KCl, 10mM DTT, 0.1mg/ml BSA, 0.5mM [^{3}H]dTTP, 0.025mM oligo(dT), 0.25mM poly(A) and 0.01% NP-40. See the unit concentration on the Product Information Label.

Usage Note: M-MLV Reverse Transcriptase is less processive than AMV Reverse Transcriptase, and therefore, more units of M-MLV enzyme are required to generate the same amount of cDNA as in the AMV reaction. Thus, starting with 1µg of mRNA in a first-strand cDNA synthesis, 200 units of the M-MLV enzyme are recommended as opposed to 25 units of AMV enzyme.

Quality Control Assays

Activity Assay

First-Strand cDNA Synthesis: 200 units of enzyme are used to produce cDNA from 1µg of 1.2kb and 6.5kb control RNAs in separate reactions, using [^{32}P] dCTP as a tracer. The minimum specification is 120ng of first-strand cDNA made from 1µg of RNA. The cDNA product must be >90% full length as determined by gel electrophoresis and autoradiography.

Contaminant Activity

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 200 units of M-MLV Reverse Transcriptase in 1X Reaction Buffer for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is <1% release for both DNase and RNase.

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 500 units of M-MLV Reverse Transcriptase in 1X Reaction Buffer for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting (analysis on 0.4µg of DNA).

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

Signed by: J. Stevens

J. Stevens, Quality Assurance

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

Part# 9PIM170

Printed in USA. Revised 2/11

Promega

Usage Information

I. Description

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (>5kb). M-MLV RT is the preferred reverse transcriptase for long mRNA templates because the RNase H activity of M-MLV RT is weaker than the commonly used Avian Myeloblastosis Virus (AMV) reverse transcriptase.

Application of M-MLV RT includes:

- First-strand synthesis of cDNA from RNA molecules

Note: M-MLV Reverse Transcriptase is less processive than AMV Reverse Transcriptase, and therefore, more units of the M-MLV enzyme are required to generate the same amount of cDNA as in the AMV reaction.

II. First-Strand Synthesis of cDNA

Materials to Be Supplied by the User

(Buffer composition is provided in Section III.)

- Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511)
- dATP, 10mM (Cat.# U1201, 100mM)
- dCTP, 10mM (Cat.# U1221, 100mM)
- dGTP, 10mM (Cat.# U1211, 100mM)
- dTTP, 10mM (Cat.# U1231, 100mM)
- Nuclease-Free Water (Cat.# P1193)

1. The following procedure uses 2µg of total RNA. In a sterile RNase-free microcentrifuge tube, add 0.5µg of the primer or primer-adaptor per microgram of the total RNA sample in a total volume of ≤15µl in water. Heat the tube to 70°C for 5 minutes to melt secondary structure within the template. Cool the tube immediately on ice to prevent secondary structure from reforming, then spin briefly to collect the solution at the bottom of the tube.
2. Add the following components to the annealed primer/template in the order shown.

   Note: Do not alter the ratio of primer to RNA.

M-MLV 5X Reaction Buffer	5µl
dATP, 10mM	1.25µl
dCTP, 10mM	1.25µl
dGTP, 10mM	1.25µl
dTTP, 10mM	1.25µl
Recombinant RNasin® Ribonuclease Inhibitor	25 units
M-MLV RT	200 units
Nuclease-Free Water to final volume	25µl

3. Mix gently by flicking the tube, and incubate for 60 minutes at 37°C for random primers or 42°C for other primers or primer-adaptors. The extension temperature may be optimized between 37°C and 42°C.
4. Perform second-strand synthesis using a protocol of your choice. Standard protocols for second-strand synthesis may be found in reference 2.

   Note: The M-MLV RT Reaction Buffer is compatible with enzymes used in a number of downstream applications. Phenol extractions and ethanol precipitations typically are not necessary before performing second-strand synthesis and amplification.

III. Composition of Buffer

M-MLV RT 5X Reaction Buffer (provided)

250mM	Tris-HCl (pH 8.3 at 25°C)
375mM	KCl
15mM	$MgCl_2$
50mM	DTT

IV. References

1. Roth, M.J., Tanese, N. and Goff, S.P. (1985) Purification and characterization of murine retroviral reverse transcriptase expressed in *Escherichia coli*. *J. Biol. Chem.* **260**, 9326–35.
2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64.

Part# 9PIM170
Printed in USA. Revised 2/11

SuperScript™ II Reverse Transcriptase

Cat. No. 18064-022 **Size: 2,000 units**
Cat. No. 18064-014 **Size: 10,000 units**
Cat. No. 18064-071 **Size: 4 × 10,000 units**

Conc. 200 U/µL **Store at –20°C (non-frost-free)**

Description

SuperScript™ II Reverse Transcriptase (RT) is an engineered version of M-MLV RT with reduced RNase H activity and increased thermal stability. The enzyme is purified to near homogeneity from *E. coli* containing the modified *pol* gene of Moloney Murine Leukemia Virus (1,2). The enzyme can be used to synthesize first-strand cDNA at higher temperatures than conventional M-MLV RT, providing increased specificity, higher yields of cDNA, and more full-length product. It can generate cDNA up to 12.3 kb.

Components

SuperScript™ II RT, 5X First-Strand Buffer (250 mM Tris-HCl, pH 8.3 at room temperature; 375 mM KCl; 15 mM $MgCl_2$), 0.1 M DTT

Storage Buffer

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) NP-40, 50% (v/v) glycerol

Storage Conditions

Store all components at –20°C in a non-frost-free freezer.
Thaw 5X First-Strand Buffer and 0.1 M DTT at room temperature just prior to use and refreeze immediately.

Unit Definition

One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10 min. at 37°C using poly(A)•oligo$(dT)_{25}$ as template-primer (3).

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Part no. 18064.pps MAN0001342 Rev. Date: 20 May 2010

First-Strand cDNA Synthesis Using SuperScript™ II RT

A 20-µL reaction volume can be used for 1 ng–5 µg of total RNA or 1–500 ng of mRNA.

1. Add the following components to a nuclease-free microcentrifuge tube:

Oligo(dT)$_{12-18}$ (500 µg/mL) *or* 50–250 ng random primers *or* 2 pmole gene-specific primer (GSP)	1 µL
1 ng to 5 µg total RNA *or* 1–500 ng of mRNA	x µL
1 µL dNTP Mix (10 mM each)	1 µL
Sterile, distilled water	to 12 µL

2. Heat mixture to 65°C for 5 min and quick chill on ice. Collect the contents of the tube by brief centrifugation and add:

5X First-Strand Buffer	4 µL
0.1 M DTT	2 µL
RNaseOUT™ (40 units/µL) (optional)*	1 µL

*RNaseOUT™ (Cat. No. 10777-019) is required if using <50 ng starting RNA.

3. Mix contents of the tube gently. If you are using oligo(dT)$_{12-18}$ or GSP, incubate at 42°C for 2 min. If you are using random primers, incubate at 25°C for 2 min.
4. Add 1 µL (200 units) of SuperScript™ II RT and mix by pipetting gently up and down.
   If you are using less than 1 ng of RNA, reduce the amount of SuperScript™ II RT to 0.25 µL (50 units) and add sterile, distilled water to a 20 µL final volume.

   If you are using random primers, incubate tube at 25°C for 10 min.
5. Incubate at 42°C for 50 min.
6. Inactivate the reaction by heating at 70°C for 15 min.

First-Strand cDNA Synthesis Using SuperScript™ II RT, Continued

The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (>1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 µL (2 units) of *E. coli* RNase H and incubate at 37°C for 20 min.

PCR

The following is intended as a guideline and starting point when using first-strand cDNA in PCR with *Taq* DNA polymerase. The optimal concentration of Mg^{++} will vary depending on the template and primer pair.

Use only 10% of the first-strand reaction for PCR. Higher volumes may not increase amplification and may result in decreased amounts of PCR product.

1. Add the following to a PCR tube:

10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]	5 µL
50 mM $MgCl_2$	1.5 µL
10 mM dNTP Mix	1 µL
Forward primer (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
Taq DNA polymerase (5 U/µL)	0.4 µL
cDNA from first-strand reaction	2 µL
autoclaved, distilled water	to 50 µL

2. Mix gently and layer with 1–2 drops (~50 µL) of silicone oil. *(Note: silicone oil is unnecessary in thermal cyclers equipped with a heated lid.)*
3. Heat reaction to 94°C for 2 min to denature.
4. Perform 15 to 40 cycles of PCR. Use the recommended annealing and extension conditions for your *Taq* DNA polymerase.

Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available at www.invitrogen.com/support.

Additional Products

RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µL) is available separately from Invitrogen (Cat. no. 10777-019).

References

1. Kotewicz, M.L., D'Alessio, J.M., Driftmier, K.M., Blodgett, K.P., and Gerard, G.F. (1985) *Gene 35*, 249.
2. Gerard, G.F., D'Alessio, J.M., Kotewicz, M.L., and Noon, M.C. (1986) *DNA 5*, 271.
3. Houts, G.E., Miyagi, M., Ellis, C., Beard, A., and Beard, J.W. (1979) *J. Virol. 29*, 517.
4. Kotewicz, M.L., Sampson, C.M., D'Alessio, J.M., and Gerard, G.F. (1988) *Nuc. Acids Res. 16*, 265.

Limited Use Label Licenses

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information about purchasing a license to use this product or the technology embedded in it for any use other than for research use please contact Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008 ; Phone (760) 603-7200 or e-mail: outlicensing@lifetech.com

invitrogen
by life technologies

SuperScript® III First-Strand Synthesis System for RT-PCR

Catalog. no.
18080-051

Size
50 reactions

Store at –30°C to –10°C

Doc. Part No 18080051.pps **Pub. No.** MAN0001346 **Rev.** 3.0

Description

The SuperScript® III First-Strand Synthesis System for RT-PCR is optimized to synthesize first-strand cDNA from purified poly(A)$^+$ or total RNA. RNA targets from 100 bp to >12 kb can be detected with this system. The amount of starting material can vary from 1 pg–5 µg of total RNA. SuperScript® III Reverse Transcriptase is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability. The enzyme is used to synthesize cDNA at a temperature range of 42–55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Because SuperScript® III RT is not significantly inhibited by ribosomal and transfer RNA, it may be used to synthesize first-strand cDNA from a total RNA preparation.

cDNA synthesis is performed in the first step using either total RNA or poly(A)$^+$-selected RNA primed with oligo(dT), random primers, or a gene-specific primer. In the second step, PCR is performed in a separate tube using primers specific for the gene of interest. For the PCR reaction, we recommend one of the following DNA polymerases: Platinum® *Taq* DNA Polymerase provides automatic hot-start conditions for increased specificity up to 4 kb, Platinum® *Taq* DNA Polymerase High Fidelity provides increased yield and high fidelity for targets up to 15 kb, and Platinum® *Pfx* DNA Polymerase provides maximum fidelity for targets up to 12 kb.

Contents

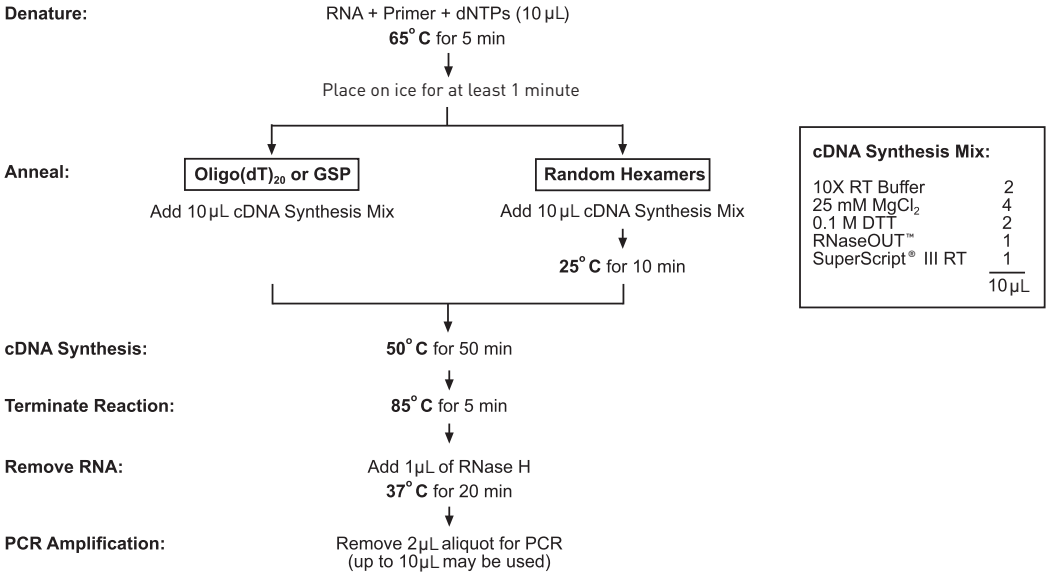
System Component	Amount
Oligo(dT)$_{20}$ (50 µM)	50 µL
Random hexamers (50 ng/µL)	250 µL
10X RT buffer*	1 mL
25 mM $MgCl_2$	500 µL
0.1 M DTT	250 µL
10 mM dNTP mix	250 µL
SuperScript® III RT (200 U/µL)	50 µL
RNaseOUT™ (40 U/µL)	100 µL
E. coli RNase H (2 U/µL)	50 µL
DEPC-treated water	1.2 mL
Total HeLa RNA (10 ng/µL)	20 µL
Sense Control Primer (10 µM)	25 µL
Antisense Control Primer (10 µM)	25 µL

*200 mM Tris-HCl (pH 8.4), 500 mM KCl

Related Products

	Amount	Catalog No.
Platinum® *Taq* DNA Polymerase	100 units	10966-018
	250 units	10966-026
	500 units	10966-034
Platinum® *Taq* DNA Polymerase High Fidelity	100 units	11304-011
	500 units	11304-029
Platinum® *Pfx* DNA Polymerase	100 units	11708-013
	250 units	11708-021
	500 units	11708-039
PCR$_x$ Enhancer System	250 rxns	11495-017
TRIzol® Reagent	100 mL	15596-026
	200 mL	15596-018
DNase I, Amplification Grade	100 units	18068-015
Custom Primers	to order, visit **www.lifetechnologies.com**	

Summary of Procedure



Recommendations and Guidelines for First-Strand Synthesis

RNA

- High-quality, intact RNA is essential for full-length, high-quality cDNA synthesis. This kit is designed for use with 1 pg–5 µg of total RNA or 1 pg–500 ng of poly(A)$^+$ RNA. For >5 µg total RNA, increase reaction volumes and amount of SuperScript® III RT proportionally.
- RNaseOUT™ Recombinant RNase Inhibitor has been added to the system to safeguard against degradation of target RNA due to ribonuclease contamination of the RNA preparation.
- To isolate total RNA, we recommend the Micro-to-Midi Total RNA Purification System (Cat. no. 12183-018), TRIzol® Reagent (Cat. nos. 15596-026/-018), or the Chomczynski and Sacchi method. Oligo (dT)-selection for poly(A)$^+$ RNA is typically not necessary, although it may improve the yield of specific cDNAs.
- Small amounts of genomic DNA in the RNA preparation may be amplified along with the target cDNA. If your application requires removal of all genomic DNA from your RNA preparation, we recommend using DNase I, Amplification Grade (Catalog no. 18068-015). DNase I, Amplification Grade, has been extensively purified to remove trace ribonuclease activities commonly associated with other "RNase-free" enzyme preparations, and does not require the addition of placental RNase inhibitor.

RNase H Digestion

The sensitivity of the PCR step can be increased (especially for long templates) by removing the RNA template from the cDNA:RNA hybrid molecule by digestion with RNase H after first-strand synthesis. Presence of RNase H during first-strand synthesis degrades the template mRNA, resulting in decreased full-length cDNA synthesis and decreased yields of first-strand cDNA. The SuperScript® III First-Strand Synthesis System introduces RNase H activity only when it is beneficial, and thus offers a unique procedural advantage over other methods.

First-Strand cDNA Synthesis

The following procedure is designed to convert 1 pg–5 µg of total RNA or 1 pg–500 ng of poly(A)$^+$ RNA into first-strand cDNA:

1. Mix and briefly centrifuge each component before use.
2. Combine the following in a 0.2- or 0.5-mL tube:

Component	Amount
up to 5 µg total RNA	*n* µL
Primer* *50 µM oligo(dT)$_{20}$, *or* 2 µM gene-specific primer (GSP), *or* 50 ng/µL random hexamers	1 µL
10 mM dNTP mix	1 µL
DEPC-treated water	to 10 µL

3. Incubate the tube at 65°C for 5 min, then place on ice for at least 1 min.
4. Prepare the following cDNA Synthesis Mix, adding each component in the indicated order.

Component	1 Rxn	10 Rxns
10X RT buffer	2 µL	20 µL
25 mM $MgCl_2$	4 µL	40 µL
0.1 M DTT	2 µL	20 µL
RNaseOUT™ (40 U/µL)	1 µL	10 µL
SuperScript® III RT (200 U/µL)	1 µL	10 µL

5. Add 10 µL of cDNA Synthesis Mix to each RNA/primer mixture, mix gently, and collect by brief centrifugation. Incubate as follows.
   Oligo(dT)$_{20}$ or GSP primed: 50 min at 50°C
   Random hexamer primed: 10 min at 25°C, followed by 50 min at 50°C
6. Terminate the reactions at 85°C for 5 min. Chill on ice.
7. Collect the reactions by brief centrifugation. Add 1 µL of RNase H to each tube and incubate the tubes for 20 min at 37°C.
8. cDNA synthesis reaction can be stored at –30°C to –10°C or used for PCR immediately.

Primers

The first-strand cDNA synthesis reaction can be primed using random hexamers, oligo(dT), or gene-specific primers (GSPs):

- Random hexamers are the most nonspecific priming method, and are typically used when the mRNA is difficult to copy in its entirety. With this method, all RNAs in a population are templates for first-strand cDNA synthesis, and PCR primers confer specificity during PCR. To maximize the size of cDNA, you should determine the ratio of random hexamers to RNA empirically for each RNA preparation.

  Note: For most RT-PCR applications, 50 ng of random hexamers per 5 µg of total RNA is adequate. Increasing hexamers to 250 ng per 5 µg of RNA may increase yield of small PCR products (<500 bp), but may decrease the yield of longer PCR products and full-length transcripts.
- Oligo(dT), a more specific priming method, is used to hybridize to 3´ poly(A) tails, which are found in the vast majority of eukaryotic mRNAs. Since poly(A)$^+$ RNA constitutes approximately 1% to 2% of total RNA, the amount and complexity of cDNA is considerably less than with random hexamers. We recommend using oligo(dT)$_{20}$ (provided in the kit).

  Note: Oligo(dT) is recommended over random hexamers or GSPs when performing RT-PCR with new mRNA targets. Oligo(dT) produces an RT-PCR product more consistently than random hexamers or GSPs.
- The most specific priming method uses a gene-specific primer for the sequence of interest. First-strand synthesis can be primed with the PCR primer that hybridizes nearest to the 3´ terminus of the mRNA. Note that some GSPs fail to prime cDNA synthesis even though they work in PCR on DNA templates. If gene-specific priming fails in RT-PCR, repeat first-strand synthesis using oligo(dT) as the primer.

Amplification of Target cDNA

The first-strand cDNA obtained in the synthesis reaction may be amplified directly using PCR. We recommend using 10% of the first-strand reaction (2 µL) for PCR. However, for some targets, increasing the amount of first-strand reaction up to 10 µL in PCR may result in increased product yield.

We recommend the following DNA polymerases (for ordering information, see page 1):

- **Platinum® *Taq* DNA Polymerase** provides automatic hot-start conditions for increased specificity and sensitivity. It is recommended for targets up to 4 kb.
- **Platinum® *Taq* DNA Polymerase High Fidelity** provides increased fidelity and higher yields for targets up to 15 kb.
- **Platinum® *Pfx* DNA Polymerase** possesses a proofreading 3´ to 5´ exonuclease activity and provides maximum fidelity for PCR. It is recommended for targets up to 12 kb.

Consult the product documentation provided with each DNA polymerase for recommended protocols and optimization guidelines. Documentation is also available at **www.lifetechnologies.com**.

Limited Product Warranty

Control Reactions

The control RNA provided with this system consists of total HeLa RNA (10 ng/µL). The sense and antisense control primers provided with this kit are designed from the human β-actin gene and produce a 353-bp RT-PCR product.

Sense primer: 5'-GCTCG TCGTC GACAA CGGCT C-3'

Antisense primer: 5'-CAAAC ATGAT CTGGG TCATC TTCTC-3'

Use the following protocol for both plus and minus RT control reactions:

1. Dilute the total HeLa RNA to 100pg/µL with DEPC-treated water.
2. Prepare the RNA/primer mixtures in sterile 0.2- or 0.5-mL tubes as follows:

Component	+ RT Control	– RT Control
Diluted total HeLa RNA (100 pg/µL)	1 µL	1 µL
Oligo(dT)$_{20}$	1 µL	1 µL
10 mM dNTP mix	1 µL	1 µL
DEPC-treated water	7 µL	7 µL

3. Incubate the samples at 65°C for 5 min, then place them on ice for at least 1 min. Collect the contents by brief centrifugation and add the following:

Component	+ RT Control	– RT Control
10X RT buffer	2 µL	2 µL
25 mM $MgCl_2$	4 µL	4 µL
0.1 M DTT	2 µL	2 µL
RNaseOUT™ (40 U/µL)	1 µL	1 µL
SuperScript® III RT (200 U/µL)	1 µL	—
DEPC-treated water	—	1 µL

4. Mix the tubes gently and collect the reactions by brief centrifugation.
5. Incubate the samples at 50°C for 50 min.
6. Terminate the reactions at 85°C for 5 min. Chill on ice.
7. Collect the reactions by brief centrifugation. Add 1 µL of RNase H to each tube and incubate for 20 min at 37°C.
8. Prepare a PCR mixture for each control reaction. For each control reaction, add the following to a 0.2-mL tube sitting on ice:

Component	Volume
DEPC-treated water	38.1 µL
10X PCR buffer minus Mg^{++}	5 µL
50 mM $MgCl_2$	1.5 µL
10 mM dNTP mix	1 µL
Control sense primer (10 µM)	1 µL
Control antisense primer (10 µM)	1 µL
cDNA from control RNA	2 µL
Taq DNA polymerase (5 units/µL)	0.4 µL
Final volume	50 µL

9. Mix the contents of the tube. Centrifuge briefly to collect the reaction components.
10. Place reaction mixture in preheated (94°C) thermal cycler. Perform an initial denaturation step: 94°C for 2 min.
11. Perform 40 cycles of PCR:

Denature	94°C for 15 sec
Anneal	55°C for 30 sec
Extend	68–72°C for 1 min

Note: For slow-ramping thermal cyclers, follow manufacturer's directions.

12. Upon completion, maintain reactions at 4°C.
13. Analyze 10 µL of each reaction, using agarose gel electrophoresis and ethidium bromide staining. For the + RT Control, a 353-bp band, corresponding to at least 25 ng of product, should be visible. For the – RT Control, the same band should be ≤50% in intensity when compared to the + RT Control.

First Strand cDNA Synthesis of Transcripts with High GC Content

High-GC content mRNAs often contain stable intrinsic secondary structures that can inhibit reverse transcriptase and/or primer annealing. Problems with RT-PCR due to this secondary structure often can be overcome by increasing the volume and temperature of the RT reaction.

Note: For templates that require cDNA synthesis temperatures above 55°C, we recommend the ThermoScript™ RT-PCR System (Catalog no. 11146-024). ThermoScript™ RT supports cDNA synthesis up to 70°C.

This protocol is suitable for gene-specific or oligo(dT) primers, but not random hexamers.

1. Mix and briefly centrifuge each component before use.
2. Prepare the RNA/primer mixture in a sterile 0.5-mL tube as follows:

Component	Sample	Control RNA
1–5 µg total RNA	*n* µL	—
Control total HeLa RNA (10 ng/µL)	—	1 µL
Oligo(dT)$_{20}$ (50 µM) *or* 2 µM GSP	1 µL	1 µL
10 mM dNTP mix	2.5 µL	2.5 µL
DEPC-treated water	to 25 µL	to 25 µL

3. Incubate each sample at 65°C for 5 min and immediately transfer to 55°C.
4. Prepare the cDNA Synthesis Mix, adding each component in the indicated order.

Component	1 Reaction	10 Reactions
DEPC-treated water	3 µL	30 µL
10X RT buffer	5 µL	50 µL
25 mM $MgCl_2$	10 µL	100 µL
0.1 M DTT	5 µL	50 µL
RNaseOUT™ Recombinant RNase Inhibitor	1 µL	10 µL
SuperScript® III RT	1 µL	10 µL

Note: For a minus RT control reaction, substitute 1 µL of DEPC-treated water for 1 µL of SuperScript® III RT, and assemble reaction as described above.

5. Prewarm the cDNA Synthesis Mix to 55°C.
6. To each sample incubating at 55°C, add 25 µL of prewarmed cDNA Synthesis Mix. Mix gently, and incubate at 55°C for 50 min.
7. Terminate the reactions at 85°C for 5 min. Chill on ice.
8. Collect the reactions by brief centrifugation. Add 1 µL of RNase H to each tube and incubate for 20 min at 37°C before proceeding to PCR.

   Note: Frequently, problems associated with RT-PCR of GC-rich cDNA are related to PCR as well as first-strand synthesis. We recommend using the PCR$_x$ Enhancer System (Catalog no. 11495-017) to facilitate amplification of GC-rich sequences.

Troubleshooting Guide

Problem	Possible Cause	Probable Solution
No bands after analysis of amplified products	Procedural error in first-strand cDNA synthesis	Use the total HeLa RNA provided as a control to verify the efficiency of the first-strand reaction (see page 3).
	RNase contamination	Add control RNA to sample to determine if RNase is present in the first-strand reaction. Maintain aseptic conditions to prevent RNase contamination. Use RNaseOUT™ Recombinant RNase Inhibitor in the first-strand reaction.
	Polysaccharide coprecipitation of RNA	Precipitate RNA with lithium chloride to remove polysaccharides, as described in Sambrook *et al.*
	Target mRNA contains strong transcriptional pauses	Use random hexamers instead of oligo(dT) in the first-strand reaction. Maintain an elevated temperature after the annealing step, as described in the protocol for cDNA synthesis from high-GC content transcripts, page 3. Increase the temperature of first-strand reaction (up to 55°C). Use PCR primers closer to the 3´ terminus of the target cDNA.
	Too little first-strand product was used in PCR	Use up to 10 µL of the first-strand reaction.
	GSP was used for first-strand synthesis	Try another GSP or switch to oligo(dT). Make sure the GSP is the antisense sequence.
	Inhibitors of RT present	Remove inhibitors by ethanol precipitation of mRNA preparation before the first-strand reaction. Include a 70% (v/v) ethanol wash of the mRNA pellet. **Note:** Inhibitors of RT include sodium dodecyl sulfate (SDS), EDTA, guanidinium salts, formamide, sodium pyrophosphate, and spermidine.
Unexpected bands after electrophoretic analysis	Contamination by genomic DNA	Pretreat RNA with DNase I, Amplification Grade (Cat. no. 18068-015), as described in the DNase I documentation. Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating genomic DNA. To test if products were derived from DNA, perform the minus RT control.
	Nonspecific annealing of primers	Vary the annealing conditions. Use Platinum® *Taq* DNA Polymerase for automatic hot-start PCR. Optimize magnesium concentration for each template and primer combination.
	Primers formed dimers	Design primers without complementary sequences at the 3´ ends.

References

Berger, S.L. and Kimmel, A.R. (1987) *Methods Enzymol* 152, 316.
Bracete, A.M., Mertz, L.M., Fox, D.K. (1999) *Focus*® 21, 38.
Chomczynski, P. (1993) *Biotechniques* Vol. 15, 532.
Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* 162, 156.
Compton, T. (1990) in *PCR Protocols: A Guide to Methods and Applications* (Innis, M., Gelfand, D., Sninsky, J., and White, T., eds.), p. 39, Academic Press, Inc.
Frohman, M.A., Dush, M.K, and Martin, G.R. (1988) *Proc. Nat. Acad. Sci USA* 85, 8998.
Gerard, G.F. (1994) *Focus*® 16, 102.
Sambrook J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
Simms, D., Cizdziel, P.E., and Chomczynski, P. (1993) *Focus*® 15, 99.
Westfall, B., Sitaraman, K., Solus, J., Hughes, J., and Rashtchian, A. (1997) *Focus*® 19, 46.
Westfall, B., Sitaraman, K., Lee, J., Borman, J. and Rashtchian, A. (1999) *Focus*® 21, 49.
Takagi, M., Nishioka, M., Kakihara, H., Kitabayashi, M., Inoue, H., Kawakami, B., Oka, M., and Imanaka, T. (1997) *Appl. Environ. Microbiol.* 63, 4504.
Sitaraman, K., Darfler, M., and Westfall, B. (1999) *Focus*® 21, 10.
Nathan, M., Mertz, L., Fox, D. (1995) *Focus*® 17, 78.
Schwabe, W., Lee, J.E., Nathan, M., Xu, R.H., Sitaraman, K., Smith, M., Potter, R.J., Rosenthal, K., Rashtchian, A., Gerard, G.F. (1998) *Focus*® 20, 30.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

ambion
by life technologies

SP6 RNA Polymerase-Plus™ Enzyme Mix

Catalog Number AM2701

Pub. No. 4393877 **Rev.** B

Contents	Quantity	Storage conditions
SP6 RNA Polymerase-Plus™ Enzyme Mix, 20 U/µL	1000 Units	Store at –20°C. *Do not store in a frost-free freezer.*
10X Transcription Buffer: 400 mM Tris pH 7.8, 200 mM NaCl, 60 mM $MgCl_2$, 20 mM Spermidine HCl, 100 mM DTT	500 µL	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

SP6 RNA Polymerase-Plus™ Enzyme Mix contains SP6 RNA polymerase and RNase inhibitor for broad-spectrum protection against enzymatic degradation of the synthesized RNA.

Source: An *E. coli* strain harboring a plasmid that overexpresses SP6 RNA Polymerase.

Unit (U) definition: One unit is the amount of SP6 RNA Polymerase-Plus™ Enzyme Mix required to catalyze the incorporation of 1 nmol of nucleoside triphosphate into acid-insoluble material in 60 minutes at 37°C.

Storage buffer (*not included*): 20 mM KPO_4, pH 7.7, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 50% glycerol (v/v), and detergent.

Using SP6 RNA Polymerase-Plus™ Enzyme Mix

SP6 RNA Polymerase-Plus™ is ideal for the synthesis of RNA for blot and *in situ* hybridization probes, RPA analysis, in vitro translation, and antisense RNA. SP6 RNA polymerase is highly specific for its own promoter, a conserved 23 bp sequence that is not efficiently recognized by T7 or T3 RNA polymerases. It will transcribe large amounts of RNA from DNA sequences (for example, plasmids, polymerase chain reaction (PCR) fragments, or hybridized oligonucleotides) downstream of its promoter, without cross-talk from nearby T7 or T3 promoters. Thus, RNA molecules transcribed from a linear template will be of a defined length. Using circular plasmid DNA as a template will result in heterogeneous transcripts of multiple lengths.

Labeled transcription reactions

The yield and amount of full-length RNA transcript obtained depend on the ratio of template DNA to the concentration of the limiting ribonucleoside triphosphate (rNTP) in the transcription reaction. Typically, three nucleotides are present at 500 µM and the nucleotide used for labeling is at various concentrations, depending on the desired specific activity of the probe (Butler and Chamberlin, 1982; Krieg and Melton, 1984). The limiting nucleotide should generally be present at a minimum of 3 µM to maximize synthesis of full-length RNA transcripts. Under standard assay conditions, >50% of the label is incorporated in 30 minutes into RNA.

We find that temperature is not a critical variable, although 37°C is frequently recommended as the appropriate incubation temperature. In fact, lower temperatures seem to favor the synthesis of full-length transcripts under conditions of limiting nucleotide concentration. It may be convenient to run these reactions at room temperature.

The following reaction conditions will yield labeled RNA probe suitable for use with Northern blots containing a moderately abundant mRNA.

Typical labeled transcription reaction conditions (20-µL reactions):

- 1 µg template DNA
- 2 µL 10X Transcription Buffer (included)
- 500 µM (final) rNTPs (A, G, C)
- 50 µCi [α-^{32}P]UTP (800 mCi/mmol, 10 mCi/mL)
- 40 U SP6 RNA Polymerase-Plus™ Enzyme Mix
- Nuclease-free water to a final volume of 20 µL

Incubate 30 minutes at 37°C.

Treat the reaction with DNase I to remove DNA template (optional; see below) or simply stop the transcription reaction by adding 2 µL of 0.2 M EDTA and/or heating to 65°C.

Unlabeled transcription reactions

In this reaction, rNTP levels are not limiting, and large amounts of RNA are synthesized throughout the incubation period. Frequently, more than 4 µg RNA may be synthesized per µg of input DNA.

Note: This protocol may be altered to include nonisotopically-labeled rNTPs. See **Technical Bulletin 173, *Methods for Nonisotopic Labeling*** for a protocol and sources of these nucleotides.

Typical unlabeled transcription reaction conditions (20-µL reaction):

- 1 µg template DNA
- 2 µL 10X Transcription Buffer (included)
- 500 µM (final) rNTPs (A, C, G, U)
- 40 U SP6 RNA Polymerase-Plus™ Enzyme Mix
- Nuclease-free water to a final volume of 20 µL

Incubate 60 minutes at 37°C.

Treat the reaction with DNase I to remove DNA template (optional; see below) or simply stop the transcription reaction by adding 2 µL of 0.2 M EDTA and/or heating to 65°C.

References

Butler, E.T. and Chamberlin, M.J. (1982) *J Biol Chemistry* 257, 5772–5778.

Krieg, P.A. and Melton, D.A. (1984) *Nucl Acids Res* 12, 7057–7070.

(Optional) Removal of DNA template

Remove the DNA template by digestion with 2 units of DNase I (RNase-free; Cat. no. AM2222, AM2224) or TURBO™ DNase (Cat. no. AM2238, AM2239) for 15 minutes at 37°C. Inactivate the DNase by adding 2 µL 0.2 M EDTA and heating at 70°C for 10 minutes, or by phenol/chloroform extraction.

For information about other post-reaction options, such as removal of unincorporated nucleotides, see the user guide for the MAXIscript® Kit (Cat. no. AM1312), available at **www.lifetechnologies.com**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

26 September 2012

ambion
by life technologies

T7 RNA Polymerase (Cloned)

Catalog Number AM2082, AM2084, AM2085

Pub. No. 4393894 **Rev.** B

Contents	Quantity	Storage conditions
T7 RNA Polymerase (Cloned)	AM2082: 5000 Units (20 U/µL) AM2084: 30,000 Units (20 U/µL) AM2085: 30,000 Units (200 U/µL)	Store at –20°C. *Do not store in a frost-free freezer.*
10X Transcription Buffer: 400 mM Tris pH 7.8, 200 mM NaCl, 60 mM $MgCl_2$, 20 mM Spermidine HCl, 100 mM DTT	500 µL	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

Cloned T7 RNA Polymerase is a high-purity T7 RNA polymerase for synthesizing high specific activity RNA probes, biologically active mRNA, and antisense RNA.

Source: An *E. coli* strain harboring a plasmid that overexpresses T7 RNA Polymerase.

Unit (U) definition: One unit is the amount of T7 RNA polymerase required to catalyze the incorporation of 1 nmol of nucleoside triphosphate into acid-insoluble material in 60 minutes at 37°C.

Storage buffer (*not included*): 20 mM KPO_4, pH 7.7, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 50% glycerol (v/v), and detergent.

Using T7 RNA Polymerase

T7 RNA polymerase is highly specific for its own promoter, a conserved 23 bp sequence that is not efficiently recognized by SP6 or T3 RNA polymerases. It will transcribe large amounts of RNA from DNA sequences (for example, plasmids, polymerase chain reaction (PCR) fragments, or hybridized oligonucleotides) downstream of its promoter, without cross-talk from nearby SP6 or T3 promoters. Thus, RNA molecules transcribed from a linear template will be of a defined length. Using circular plasmid DNA as a template will result in heterogeneous transcripts of multiple lengths.

Labeled transcription reactions

The yield and amount of full-length RNA transcript obtained depend on the ratio of template DNA to the concentration of the limiting ribonucleoside triphosphate (rNTP) in the transcription reaction. Typically, three nucleotides are present at 500 µM and the nucleotide used for labeling is at various concentrations, depending on the desired specific activity of the probe (Tabor and Richardson, 1985). The limiting nucleotide should generally be present at a minimum of 3 µM to maximize synthesis of full-length RNA transcripts. Under standard assay conditions, >50% of the label is incorporated in 30 minutes into RNA.

We find that temperature is not a critical variable, although 37°C is frequently recommended as the appropriate incubation temperature. In fact, lower temperatures seem to favor the synthesis of full-length transcripts under conditions of limiting nucleotide concentration. It may be convenient to run these reactions at room temperature.

The following reaction conditions will yield labeled RNA probe suitable for use with Northern blots containing a moderately abundant mRNA.

Typical labeled transcription reaction conditions (20-µL reactions):

- 1 µg template DNA
- 2 µL 10X Transcription Buffer (included)
- 500 µM (final) rNTPs (A, G, C)
- 50 µCi [α-^{32}P]UTP (800 mCi/mmol, 10 mCi/mL)
- 0.1–1.0 U/µL (final) RNase Inhibitor (Cat. no. AM2682, AM2684)
- 20 U T7 RNA Polymerase
- Nuclease-free water to a final volume of 20 µL

Incubate 30 minutes at 37°C.

Treat the reaction with DNase I to remove DNA template (optional; see below) or simply stop the transcription reaction by adding 2 µL of 0.2 M EDTA and/or heating to 65°C.

Unlabeled transcription reactions

In this reaction, rNTP levels are not limiting, and large amounts of RNA are synthesized throughout the incubation period. Frequently, more than 4 µg RNA may be synthesized per µg of input DNA.

Note: This protocol may be altered to include nonisotopically-labeled rNTPs. See **Technical Bulletin 173, *Methods for Nonisotopic Labeling*** for a protocol and sources of these nucleotides.

Typical unlabeled transcription reaction conditions (20-µL reaction):

- 1 µg template DNA
- 2 µL 10X Transcription Buffer (included)
- 500 µM (final) rNTPs (A, C, G, U)
- 0.1–1.0 U/µL (final) RNase Inhibitor (Cat. no. AM2682, AM2684)
- 20 U T7 RNA Polymerase
- Nuclease-free water to a final volume of 20 µL

Incubate 60 minutes at 37°C.

Treat the reaction with DNase I to remove DNA template (optional; see below) or simply stop the transcription reaction by adding 2 µL of 0.2 M EDTA and/or heating to 65°C.

(Optional) Removal of DNA template

Remove the DNA template by digestion with 2 units of DNase I (RNase-free; Cat. no. AM2222, AM2224) or TURBO™ DNase (Cat. no. AM2238, AM2239) for 15 minutes at 37°C. Inactivate the DNase by adding 2 µL 0.2 M EDTA and heating at 70°C for 10 minutes, or by phenol/chloroform extraction.

For information about other post-reaction options, such as removal of unincorporated nucleotides, see the user guide for the MAXIscript® Kit (Cat. no. AM1312), available at **www.lifetechnologies.com**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

References

Tabor, S. and Richardson, C.C. (1985) *Proc. Natl. Acad. Sci. USA* 82, 1074–1078.

Certificate of Analysis

Part# 9PIM180

Revised 7/13

T4 DNA Ligase:

Part No.	Size (Weiss units)
M180A	100
M180B	500
M179A	(High Conc.) 500

Ligase Buffer, 10X (C126A, C126B): The Ligase 10X Buffer supplied with this enzyme has a composition of 300mM Tris-HCl (pH 7.8), 100mM $MgCl_2$, 100mM DTT and 10mM ATP. The performance of this buffer depends on the integrity of the ATP. Store the buffer in small aliquots at –20°C to minimize degradation of the ATP and DTT.

Note: The DTT in the Ligase 10X Buffer may precipitate upon freezing. If this occurs, vortex the buffer until the precipitate is in solution (typically 1–2 minutes). The performance of the product is not affected provided that the precipitate is resuspended.

Enzyme Storage Buffer: T4 DNA Ligase is supplied in 10mM Tris-HCl (pH 7.4), 50mM KCl, 1mM DTT, 0.1mM EDTA and 50% glycerol.

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

Unit Definition: 0.01 Weiss unit of T4 DNA Ligase is defined as the amount of enzyme required to catalyze the ligation of greater than 95% of the *Hin*d III fragments of 1µg of Lambda DNA at 16°C in 20 minutes. See the unit concentration on the Product Information Label.

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

Quality Control Assays

Activity Assays

Blue/White Assay: pGEM®-3Zf(+) Vector is digested with representative restriction enzymes (leaving 5´-termini, 3´-termini or blunt ends). Each microgram of cut plasmid is ligated with 4 units of T4 DNA Ligase. The DNA is then transformed into JM109 cells that are plated on X-Gal/IPTG/Ampicillin plates. White colonies result from transformation with ligated plasmids with damaged ends. These white colonies represent the number of false positives expected in a typical cloning experiment. Enzymes that generate overhangs must produce fewer than 2% white colonies, and blunt-cutting enzymes must produce fewer than 5% white colonies.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 20 units of T4 DNA Ligase in 1X Ligase Buffer (Stock# C126 at 1X) for 16 hours at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel. There must be no visible nicking or cutting of the DNA.

Single-Stranded and Double-Stranded DNase Assay: To test for DNase activity, 50ng of radiolabeled single-stranded or double-stranded DNA is incubated with 20 units of T4 DNA Ligase in 1X Ligase Buffer (Stock# C126 at 1X) for 16 hours at 37°C. Minimum passing specification is <2% release of single-stranded and <1% release of double-stranded radiolabeled nucleotides as monitored by scintillation counting of TCA-soluble material.

RNase Assay: To test for RNase activity, 50ng of radiolabeled RNA is incubated with 20 units of T4 DNA Ligase in 1X Ligase Buffer (Stock# C126 at 1X) for 5 hours at 37°C. Minimum passing specification is <3% release of radiolabeled nucleotides as monitored by scintillation counting of TCA-soluble material.

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

Signed by: J. Stevens

J. Stevens, Quality Assurance

AF9PI M180 0713M180

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

Part# 9PIM180
Printed in USA. Revised 7/13

Promega

Usage Information

I. Description

T4 DNA Ligase catalyzes the joining of two strands of DNA between the 5´-phosphate and the 3´-hydroxyl groups of adjacent nucleotides in either a cohesive-ended or blunt-ended configuration (1). The enzyme has also been shown to catalyze the joining of RNA to either a DNA or RNA strand in a duplex molecule but will not join single-stranded nucleic acids (1).

II. Standard Applications

A. Ligation of DNA

Material to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)

We recommend using a 1:1, 1:3 or 3:1 molar ratio of vector:insert DNA when cloning a fragment into a plasmid vector. These ratios will vary with other types of vectors, for example, cDNA and genomic cloning vectors. The following example illustrates the conversion of molar ratios to mass ratios for a 3.0kb plasmid and a 0.5kb insert DNA fragment.

$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

Example:

How much 0.5kb insert DNA should be added to a ligation in which 100ng of 3kb vector will be used? The desired vector:insert ratio will be 1:3.

$$\frac{100\text{ng vector} \times 0.5\text{kb insert}}{3\text{kb vector}} \times \frac{3}{1} = 50\text{ng insert}$$

The following ligation reaction of a 3kb vector and a 0.5kb insert DNA uses a 1:1 vector:insert ratio. Typical ligation reactions use 100–200ng of vector DNA.

1. Assemble the following reaction in a sterile microcentrifuge tube:

vector DNA	100ng
insert DNA	17ng
Ligase 10X Buffer	1µl
T4 DNA Ligase (Weiss units)	0.1–1u
Nuclease-Free Water to final volume of	10µl

2. Incubate the reaction at:

   room temperature for 3 hours, or
   4°C overnight, or
   15°C for 4–18 hours.

Notes:

1. There is considerable latitude in the temperature and time needed for successful ligations. The optimal temperature for a ligation is a balance between the optimal temperature for T4 DNA Ligase enzyme activity (25°C) (1) and the temperature necessary to ensure annealing of the fragment ends, which can vary with the length and base composition of the overhangs. Shorter duplexes (linkers less than 16 bases long) require lower temperatures as a result of their lower melting temperatures (T_m). In general, ligation reactions performed at lower temperatures require longer incubation times. The scientific literature reflects this variability in ligation conditions. Blunt-end ligations generally are efficient at temperatures between 15–20°C for 4–18 hours, while sticky ends are ligated effectively at room temperature (22°C) for 3 hours or 4–8°C overnight.
2. The ligation conditions given in this protocol are based on the conditions used at Promega for quality control of lambda vectors with sticky ends. These ligation conditions have been developed using Promega Blue/White Cloning-Qualified T4 DNA Ligase.
3. The addition of polyethylene glycol (PEG) to ligation reactions can promote ligation of blunt-ended fragments by "macromolecular crowding" (2). We do not recommend the use of PEG in ligations, however, due to extreme variability in the quality of PEG. In addition, the use of PEG can lead to undesirable concatemerization when cloning cDNAs, and residual PEG is inhibitory to lambda packaging reactions.

III. Additional Information

Molecular Weight: 68kDa (3).
Requirements: Mg^{2+}, ATP and DTT (3). The optimum concentration of Mg^{2+} is 10mM. Mn^{2+} may be substituted for Mg^{2+} but is only 25% as effective as Mg^{2+} (1).
Inhibition: 50% inhibition by greater than 150mM NaCl (activity measured at nicks) (3). Other inhibitors include 0.2M K^+, Cs^+, Li^+, NH_4^+ and 1mM spermine (1).
Inactivation: Heat to 70°C for 10 minutes (4).

IV. References

1. Engler, M.J. and Richardson, C.C. (1982) In: *The Enzymes*, Boyer, P.D., ed., Academic Press, New York, NY.
2. Zimmerman, S.B. and Pheiffer, B.H. (1983) Macromolecular crowding allows blunt-end ligation by DNA ligases from rat liver or *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **80**, 5852–6.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. *Protocols and Applications Guide*, Third Edition (1996) Promega Corporation.

Part# 9PIM180
Printed in USA. Revised 7/13

Technical Bulletin

T4 Polynucleotide Kinase

INSTRUCTIONS FOR USE OF PRODUCTS M4101 AND M4103.

www.promega.com

PRINTED IN USA.
Revised 6/09

Part# TB519

T4 Polynucleotide Kinase

All technical literature is available on the Internet at: www.promega.com/tbs/ Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

1. Description

T4 Polynucleotide Kinase (T4 PNK) catalyzes the transfer of the γ-phosphate from ATP to the 5´-terminus of polynucleotides or to mononucleotides bearing a 3´-phosphate group (1). T4 PNK is widely used to end-label short oligonucleotide probes (2), DNA (3) and RNA (4) molecules. Under certain conditions, the reaction with polynucleotides can be made reversible, permitting exchange of the γ-phosphate of ATP with the 5´ terminal phosphate of a polynucleotide, thus circumventing the need to dephosphorylate the substrate with alkaline phosphatase (5).

2. Product Components and Storage Conditions

Product	Size	Cat.#
T4 Polynucleotide Kinase	100 units	M4101
	1,000 units	M4103

For Laboratory Use. Cat.# M4101 includes:

- 1 vial T4 Polynucleotide Kinase
- 1 vial Kinase 10X Reaction Buffer

Storage Conditions: Store the T4 Polynucleotide Kinase and the Kinase 10X Reaction Buffer at –20°C.

3. Standard Applications

3.A. 5´ End-Labeling Protocol, Forward Reaction

Materials to Be Supplied by the User
(Solution compositions are provided in Section 5.)

- 0.5M EDTA
- ethanol, 100%
- phenol:chloroform:isoamyl alcohol (25:24:1)
- TE buffer
- 7.5M ammonium acetate or 3M sodium acetate (pH 5.5)
- [γ-^{32}P]ATP (at 3,000Ci/mmol, 10mCi/ml)

Dephosphorylate the DNA to be labeled prior to performing the labeling reaction. Labeling of protruding 5´ ends is more efficient than the labeling of blunt ends or recessed 5´ ends. Alternative protocols may be used to increase the labeling efficiency of these templates (6,7).

1. Assemble the following reaction in a sterile microcentrifuge tube:

5´ ends of DNA (dephosphorylated)	1–50pmol
Kinase 10X Buffer	5µl
[γ-^{32}P]ATP (at 3,000Ci/mmol, 10mCi/ml, 50pmol total)	15µl
T4 Polynucleotide Kinase	10–20u
deionized water to a final volume of	50µl

! Do not dissolve DNA in, or precipitate from, buffers containing ammonium salts prior to treatment with T4 PNK (6). Ammonium ions are strong inhibitors of T4 Polynucleotide Kinase.

2. Incubate at 37°C for 10 minutes.
3. Stop the reaction by adding 2µl of 0.5M EDTA.
4. Add one volume of phenol:chloroform:isoamyl alcohol (25:24:1). Vortex for 1 minute and spin at full speed in a microcentrifuge for 2 minutes.
5. Transfer the upper, aqueous phase to a fresh tube. Add one volume of chloroform:isoamyl alcohol (24:1). Vortex for 1 minute and spin at full speed in a microcentrifuge for 2 minutes.
6. Transfer the upper, aqueous phase to a fresh tube. Add 0.5 volume of 7.5M ammonium acetate or 0.1 volume of 3M sodium acetate.
7. Add 2 volumes of 100% ethanol, mix and place at –70°C for 30 minutes.
8. Spin at full speed in a microcentrifuge for 5 minutes.
9. Decant the supernatant, and resuspend the DNA in 50µl of TE buffer.

The labeled DNA can be separated from unincorporated labeled nucleotides with the Wizard® DNA Clean-Up System (Cat.# A7280), chromatography or an additional ethanol precipitation.

3.B. Non-Radioactive Kinase Reaction (8)

Materials to Be Supplied by the User
(Solution compositions are provided in Section 5.)

- 0.5M EDTA
- ethanol, 100%
- phenol:chloroform:isoamyl alcohol (25:24:1)
- TE buffer
- 7.5M ammonium acetate or 3M sodium acetate (pH 5.5)
- 0.1mM ATP stock

Phosphorylation of insert DNA may be required for ligation with a nonphosphorylated vector. The following reaction may be used for non-radioactive phosphorylation of insert DNA.

1. Assemble the following reaction components in a sterile tube:

insert DNA (average size of 1.5kb)	250ng
Kinase 10X Buffer	4µl
0.1mM ATP	2µl
T4 Polynucleotide Kinase	10–20u
deionized water to a final volume of	40µl

   Note: For the non-radioactive labeling of oligonucleotides, use 100pmol of oligo and increase the ATP to a final concentration of 1mM in Step 1.

2. Incubate at 37°C for 30 minutes.
3. Stop the reaction by adding 2µl of 0.5M EDTA.
4. Phenol extract and ethanol precipitate as in Section 3.A, Steps 4–8.
5. Decant the supernatant, and resuspend the DNA in 50µl of TE buffer.

3.C. Exchange Reaction Using Protruding 5´ Phosphoryl Termini as Templates (6)

Materials to Be Supplied by the User
(Solution compositions are provided in Section 5.)

- exchange reaction 10X buffer
- 100% ethanol
- phenol:chloroform:isoamyl alcohol (25:24:1)
- chloroform:isoamyl alcohol (24:1)
- 5mM ADP
- 7.5M ammonium acetate or 3M sodium acetate (pH 5.5)
- [γ-^{32}P]ATP (at 3,000Ci/mmol, 10mCi/ml)
- 0.5M EDTA
- TE buffer

The exchange reaction of T4 Polynucleotide Kinase is much less efficient than the forward reaction described above. Also, the buffer conditions for the exchange reaction are different, so the provided Kinase 10X Buffer cannot be used.

1. Assemble the following reaction in a sterile microcentrifuge tube:

5´ ends of DNA (5´ phosphorylated)	1–50pmol
exchange reaction 10X buffer	5µl
5mM ADP	3µl
[γ-^{32}P]ATP (at 3,000Ci/mmol, 10mCi/ml, 100pmol total)	30µl
T4 Polynucleotide Kinase	20u
deionized water to a final volume of	50µl

2. Incubate at 37°C for 30 minutes.
3. Stop the reaction by adding 2µl of 0.5M EDTA.
4. Phenol extract and ethanol precipitate as in Section 3.A, Steps 4–8.
5. Decant the supernatant, and resuspend the DNA in 50µl of TE buffer.

The labeled DNA may be separated from unincorporated labeled nucleotides with our Wizard® DNA Clean-Up System (Cat.# A7280), chromatography or an additional ethanol precipitation.

Notes:

1. 1 mole of 5´ ends = 0.5 mole of DNA (6).
2. The final ATP concentration in these reactions should be at least 1µM (6).

4. Product Specifications

Source: T4 PNK is purified from *E. coli* cells expressing a recombinant clone.

Molecular Weight: 132kDa. T4 PNK is a tetramer of identical monomers of apparent molecular weight of 33kDa (9).

Unit Definition: One unit is defined as the amount of T4 Polynucleotide Kinase required to catalyze the transfer of 1nmol of phosphate to the 5´-OH end of a polynucleotide from [γ-^{32}P]ATP in 30 minutes at 37°C.

Inhibitor: Ammonium ions and low levels of phosphate buffers (7).

Optimum pH Range: 7.4 to 8.0 in Tris-HCl buffer, with optimal activity obtained at pH 7.6 (10).

Maximum Activity: Obtained at 37°C (pH 7.6) in the presence of 10mM Mg^{2+} and thiol reagents (5mM DTT or 10mM 2-mercaptoethanol), with a minimum of 1µM ATP and 5:1 ratio of ATP over 5´-OH ends (9,10).

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

Unit Definition Assay Buffer: 40mM Tris-HCl (pH 7.5), 10mM $MgCl_2$, 5mM DTT, 0.1mM [γ-^{32}P]ATP and 0.5µg/µl 5´-OH polynucleotide end concentration.

Storage Buffer: T4 Polynucleotide Kinase is supplied in 20mM Tris-HCl (pH 7.5), 25mM KCl, 2mM DTT, 0.1mM EDTA, 0.1µM ATP, 50% (v/v) glycerol.

5. Composition of Buffers and Solutions

exchange reaction 10X buffer

500mM	imidazole-HCl (pH 6.6)
100mM	$MgCl_2$
50mM	DTT
1mM	spermidine
1mM	EDTA

TE buffer

10mM	Tris-HCl (pH 8.0)
1mM	EDTA

phenol:chloroform:isoamyl alcohol (25:24:1)

Mix equal parts of TE buffer and phenol, and allow the phases to separate. Then mix 1 part of the lower, phenol phase with 1 part of chloroform:isoamyl alcohol (24:1).

6. References

1. Richardson, C.C. (1965) Phosphorylation of nucleic acid by an enzyme from T4 bacteriophage-infected *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **54**, 158–65.
2. Agarwal, K. *et al.* (1981) A general method for detection and characterization of an mRNA using an oligonucleotide probe. *J. Biol. Chem.* **256**, 1023–8.
3. Maxam, A.M. and Gilbert, W. (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**, 499–560.
4. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Mapping adenines, guanines, and pyrimidines in RNA. *Nucleic Acids Res.* **4**, 2527–38.
5. Berkner, K.L. and Folk, W.R. (1977) Polynucleotide kinase exchange reaction: Quantitave assay for restriction endonuclease-generated 5´-phosphoryl termini in DNA. *J. Biol. Chem.* **252**, 3176–84.
6. Maniatis, T. *et al.* (1982) *Molecular Cloning: A Laboratory Manua*l, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 122.
7. Ausubel, F.M. *et al.* (1987) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 3.10.4–3.10.5.
8. *Protocols and Applications Guide,* Third Edition (1996) Promega Corporation, 186.
9. Perbal, B. (1988) *A Practical Guide to Molecular Cloning*, 2nd edition, John Wiley and Sons, 86.
10. *The Enzymes* (1981) Vol. XIV, Nucleic Acids, Part A, 3rd edition, P.D. Boyer, ed., Academic Press, 308.

Antarctic Phosphatase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0289S 019120414041

M0289S

RR 37° Yes

1,000 units	**5,000 U/ml**	**Lot: 0191204**
RECOMBINANT	**Store at –20°C**	**Exp: 4/14**

Description: Antarctic Phosphatase catalyzes the removal of 5´ phosphate groups from DNA and RNA. Since phosphatase-treated fragments lack the 5´ phosphoryl termini required by ligases, they cannot self-ligate (1). This property can be used to decrease the vector background in cloning strategies.

Heat Inactivated In 5 Minutes at 65°C

Source: An *E. coli* strain that carries the TAB5 AP gene, originally cloned in plasmid pNI (2), recloned in plasmid pEGTAB7–4.1(3).

Applications:

- Removing 5´ phosphates from DNA, RNA, rNTPs and dNTPs
- Preparation of templates for 5´ end labeling
- Prevention of recircularization of cloning vectors
- Dephosphorylation of proteins
- Removal of dNTPs and pyrophosphate from PCR reactions

Supplied in: 10 mM Tris-HCl (pH 7.4), 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X Antarctic Phosphatase Reaction Buffer.

Reaction Conditions: 1X Antarctic Phosphatase Reaction Buffer. Incubate at 37°C.

1X Antarctic Phosphatase Reaction Buffer:
50 mM Bis-Tris-Propane HCl
1 mM $MgCl_2$
0.1 mM $ZnCl_2$
pH 6.0 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will dephosphorylate 1 µg of pUC19 vector DNA cut with HindIII (5´ protruding ends), HincII (blunts ends) or Pst I (5´ recessed ends) in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recirculation in a self-ligation reaction and is measured by transformation into *E. coli.*

Unit Assay Conditions: Vector DNA is dephosphorylated in restriction endonuclease buffer supplemented with Antarctic Phosphatase Reaction Buffer. Ligation is performed with 50 ng of vector using the NEB Quick Ligation Kit (NEB #M2200).

Vector Dephosphorylation Protocol:

1. Add 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer to 1– 5 µg of DNA cut with any restriction endonuclease in any buffer.
2. Add 1 µl of Antarctic Phosphatase (5 units) and mix.
3. Incubate for 15 minutes at 37°C for 5´ extensions or blunt-ends, 60 minutes for 3´ extensions.
4. Heat inactivate for 5 minutes at 65°C (or as required to inactivate the restriction enzyme).
5. Proceed with ligation.

Usage Notes: Antarctic Phosphatase is also active in NEBuffers 1, 2, 3 or 4 as well as the NEBuffer for EcoRI **ONLY** when supplemented with 10X Antarctic Phosphatase Reaction Buffer to a final concentration of 1X. Adding 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer will provide the amount of Zn^{2+} that the enzyme requires for activity.

Quality Control Assays
Exonuclease Activity: Incubation of a 50 µl reaction containing 50 units of Antarctic Phosphatase with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of Antarctic Phosphatase with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 50 units of Antarctic Phosphatase with 40 ng of fluorescein labeled RNA transcript for 4 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis using fluorescence detection.

CERTIFICATE OF ANALYSIS

Antarctic Phosphatase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0289S 019120414041

M0289S

RR 37° Yes

1,000 units	**5,000 U/ml**	**Lot: 0191204**
RECOMBINANT	**Store at –20°C**	**Exp: 4/14**

Description: Antarctic Phosphatase catalyzes the removal of 5´ phosphate groups from DNA and RNA. Since phosphatase-treated fragments lack the 5´ phosphoryl termini required by ligases, they cannot self-ligate (1). This property can be used to decrease the vector background in cloning strategies.

Heat Inactivated In 5 Minutes at 65°C

Source: An *E. coli* strain that carries the TAB5 AP gene, originally cloned in plasmid pNI (2), recloned in plasmid pEGTAB7–4.1(3).

Applications:

- Removing 5´ phosphates from DNA, RNA, rNTPs and dNTPs
- Preparation of templates for 5´ end labeling
- Prevention of recircularization of cloning vectors
- Dephosphorylation of proteins
- Removal of dNTPs and pyrophosphate from PCR reactions

Supplied in: 10 mM Tris-HCl (pH 7.4), 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X Antarctic Phosphatase Reaction Buffer.

Reaction Conditions: 1X Antarctic Phosphatase Reaction Buffer. Incubate at 37°C.

1X Antarctic Phosphatase Reaction Buffer:
50 mM Bis-Tris-Propane HCl
1 mM $MgCl_2$
0.1 mM $ZnCl_2$
pH 6.0 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will dephosphorylate 1 µg of pUC19 vector DNA cut with HindIII (5´ protruding ends), HincII (blunts ends) or Pst I (5´ recessed ends) in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recirculation in a self-ligation reaction and is measured by transformation into *E. coli.*

Unit Assay Conditions: Vector DNA is dephosphorylated in restriction endonuclease buffer supplemented with Antarctic Phosphatase Reaction Buffer. Ligation is performed with 50 ng of vector using the NEB Quick Ligation Kit (NEB #M2200).

Vector Dephosphorylation Protocol:

1. Add 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer to 1– 5 µg of DNA cut with any restriction endonuclease in any buffer.
2. Add 1 µl of Antarctic Phosphatase (5 units) and mix.
3. Incubate for 15 minutes at 37°C for 5´ extensions or blunt-ends, 60 minutes for 3´ extensions.
4. Heat inactivate for 5 minutes at 65°C (or as required to inactivate the restriction enzyme).
5. Proceed with ligation.

Usage Notes: Antarctic Phosphatase is also active in NEBuffers 1, 2, 3 or 4 as well as the NEBuffer for EcoRI **ONLY** when supplemented with 10X Antarctic Phosphatase Reaction Buffer to a final concentration of 1X. Adding 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer will provide the amount of Zn^{2+} that the enzyme requires for activity.

Quality Control Assays
Exonuclease Activity: Incubation of a 50 µl reaction containing 50 units of Antarctic Phosphatase with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of Antarctic Phosphatase with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 50 units of Antarctic Phosphatase with 40 ng of fluorescein labeled RNA transcript for 4 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis using fluorescence detection.

CERTIFICATE OF ANALYSIS

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Transformation Assay: pUC19 was cleaved with HindIII, HincII or PstI, each purified by Qiaprep™ spin column and resuspended in water at 0.2 mg/ml. 1 µg of each DNA was treated with 5 units of Antarctic Phosphatase. Each reaction was carried out with 1 µg of DNA in a 50 µl reaction volume with 1X Antarctic Phosphatase Reaction Buffer for 30 minutes at 37°C, followed by heat inactivation at 65°C for 5 minutes.

Ligations were performed using the NEB Quick Ligation Kit protocol with 2.5 µl (50 ng) of vector DNA directly from the heat-inactivated phosphatase reaction mix. Inserts were included in 3-fold molar excess. Either HindIII cleaved fragments of λ DNA, HaeIII cleaved fragments of ϕX174 RF I DNA or PstI cleaved fragments of λ DNA were inserted as indicated.

5 ng of each ligation was transformed into *E. coli* DH5-α. The equivalent of 1.0 ng were plated on LB plates that contained IPTG, X-gal and ampicillin.

	Antarctic Phosphatase Units	Blue Colonies	White Colonies	Background Reduction (%)	Insert (%)
5´ Overhang					
HindIII cleaved and ligated	0	1360	0		
Phosphatase treated and ligated	5	16	0		
Phosphatase treated, insert ligated	5	24	216	98.2	15.9
Blunt End					
HincII cleaved and ligated	0	1028	0		
Phosphatase treated and ligated	5	0	0		
Phosphatase treated, insert ligated	5	24	92	97.7	8.9
3´ Overhang					
PstI cleaved and ligated	0	872	0		
Phosphatase treated and ligated	5	8	0		
Phosphatase treated, insert ligated	5	20	348	97.7	40.0

Heat Inactivation: 65°C for 5 minutes.

References:

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning*: *A Laboratory Manual*, (2nd ed.), (p. 5.72). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Rina, M. et al. (2000) *Eur. J. Biochem.* 267, 1230–1238.
3. Guthrie, E., unpublished observations

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Transformation Assay: pUC19 was cleaved with HindIII, HincII or PstI, each purified by Qiaprep™ spin column and resuspended in water at 0.2 mg/ml. 1 µg of each DNA was treated with 5 units of Antarctic Phosphatase. Each reaction was carried out with 1 µg of DNA in a 50 µl reaction volume with 1X Antarctic Phosphatase Reaction Buffer for 30 minutes at 37°C, followed by heat inactivation at 65°C for 5 minutes.

Ligations were performed using the NEB Quick Ligation Kit protocol with 2.5 µl (50 ng) of vector DNA directly from the heat-inactivated phosphatase reaction mix. Inserts were included in 3-fold molar excess. Either HindIII cleaved fragments of λ DNA, HaeIII cleaved fragments of ϕX174 RF I DNA or PstI cleaved fragments of λ DNA were inserted as indicated.

5 ng of each ligation was transformed into *E. coli* DH5-α. The equivalent of 1.0 ng were plated on LB plates that contained IPTG, X-gal and ampicillin.

	Antarctic Phosphatase Units	Blue Colonies	White Colonies	Background Reduction (%)	Insert (%)
5´ Overhang					
HindIII cleaved and ligated	0	1360	0		
Phosphatase treated and ligated	5	16	0		
Phosphatase treated, insert ligated	5	24	216	98.2	15.9
Blunt End					
HincII cleaved and ligated	0	1028	0		
Phosphatase treated and ligated	5	0	0		
Phosphatase treated, insert ligated	5	24	92	97.7	8.9
3´ Overhang					
PstI cleaved and ligated	0	872	0		
Phosphatase treated and ligated	5	8	0		
Phosphatase treated, insert ligated	5	20	348	97.7	40.0

Heat Inactivation: 65°C for 5 minutes.

References:

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning*: *A Laboratory Manual*, (2nd ed.), (p. 5.72). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Rina, M. et al. (2000) *Eur. J. Biochem.* 267, 1230–1238.
3. Guthrie, E., unpublished observations

Page 2 (M0289)

Certificate of Analysis

Terminal Deoxynucleotidyl Transferase, Recombinant:

Part No.	Size (units)
M828A	300
M828C	1,500

Part# 9PIM187
Revised 9/13

Description: This enzyme catalyzes the repetitive addition of mononucleotides to the terminal 3´-OH of a DNA initiator accompanied by the release of inorganic phosphate. Single-stranded DNA is preferred as an initiator. Polymerization is not template-dependent. The addition of 1mM Co^{2+} (as $CoCl_2$) in the reaction buffer allows the tailing of 3´-ends with varying degrees of efficiency.

Enzyme Storage Buffer: Terminal Deoxynucleotidyl Transferase, Recombinant, is supplied in 50mM potassium phosphate (pH 6.4), 100mM NaCl, 1mM β-mercaptoethanol, 0.1% Tween® 20 and 50% glycerol.

Source: Recombinant *E. coli* strain.

Storage Conditions: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Terminal Transferase 5X Buffer (M189A): 500mM cacodylate buffer (pH 6.8), 5mM $CoCl_2$ and 0.5mM DTT.

Unit Definition: One unit of activity catalyzes the transfer of 0.5 picomoles of ddATP to oligo$(dT)_{16}$ per minute at 37°C in 1X Terminal Transferase Buffer. The resulting oligo$(dT)_{17}$ is measured by HPLC.

Usage Notes for 3´-End Labeling Reaction

1. Not all dNTPs are tailed with the same efficiency. Actual concentration of dNTP will depend on the individual application.
2. The provided buffer (5X) is to be used in the tailing reaction. The recommended reaction conditions are as described under Quality Control Assays, 3´-End Labeling Reaction, and in Section III overleaf.

Quality Control Assays

Functional Assays

3´-End Labeling Reaction: Two micromolar oligo$(dT)_{16}$ is incubated in 1X Terminal Transferase Buffer together with 40µM ddATP and 1 unit of Terminal Deoxynucleotidyl Transferase (in a final volume of 50µl) for 30 minutes at 37°C. The specification is that >50% of the oligo$(dT)_{16}$ is converted to oligo$(dT)_{17}$.

TUNEL Assay: HL-60 cells at a concentration of 5×10^5 cells/ml are treated with anisomycin in DMSO and incubated at 37°C for 2 hours. The cells are fixed onto microscope slides and processed for TUNEL staining using 30 units of Terminal Deoxynucleotidyl Transferase, Recombinant, and the DeadEnd™ Fluorometric TUNEL System (Cat.# G3250). The procedure is outlined in Technical Bulletin #TB235 (www.promega.com/tbs/tb235/tb235.html)

Contaminant Activity

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 25 units of Terminal Deoxynucleotidyl Transferase, Recombinant, in 1X Reaction Buffer plus 10mM $MgCl_2$ for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. The specification is <3% release for both DNase and RNase.

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 5 units of Terminal Deoxynucleotidyl Transferase, Recombinant, in 1X Reaction Buffer plus 10mM $MgCl_2$ for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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

Part# 9PIM187
Printed in USA. Revised 9/13

Promega

Usage Information

I. Description

Terminal Deoxynucleotidyl Transferase, Recombinant (rTdT), is an enzyme that catalyzes the repetitive addition of mononucleotides from dNTPs to the terminal 3´-OH of a DNA initiator, accompanied by the release of inorganic phosphate (1). The enzyme thus provides a unique method for the labeling of the 3´ termini of DNA.

II. Product Components

Product	Size	Cat.#
Terminal Deoxynucleotidyl Transferase, Recombinant	300 units	M1871
	1,500 units	M1875

In addition to Terminal Deoxynucleotidyl Transferase, M1871 and M1875 also include:

- 500µl Terminal Transferase 5X Buffer

III. Addition of [α-^{32}P]dNTP to the 3´ Termini of Single-Stranded DNA Primers

1. Set up the following reaction:

Terminal Transferase 5X Buffer	4.0µl
primer	2pmol
[α-^{32}P]dATP (800Ci/mmol, 10mCi/ml)	1.6µl
Terminal Deoxynucleotidyl Transferase, Recombinant	10–20 units
water to a final volume of	20µl

2. Incubate at 37°C for 60 minutes.

3. Stop the reaction by heating at 70°C for 10 minutes.

Table 1. Amount of DNA Primer (ng) Needed to Equal 2pmol.

Primer Length	Amount (ng) of Primer Equal to 2pmol
15mer	10ng
18mer	12ng
24mer	16ng
31mer	21ng

In general, ng of primer = pmol of primer × 0.33 × N, where N = length of primer in bases.

Notes:

1. The length and distribution of the homopolymer tails added by TdT depends on several factors including the nucleotide used, substrate concentrations, ratio of DNA primer to nucleotide and reaction time and temperature. Reference 2 contains a discussion of the factors affecting the length and distribution of homopolymer tails generated by native TdT.

2. The enzyme-to-substrate ratio is also critical for obtaining uniform addition of labeled nucleotides. Therefore, 10–20 units of enzyme are recommended for 2–4pmol of substrate DNA. Ratios lower than this produce probes of varying lengths and reduce incorporation rates.

3. Reactions can be carried out overnight without affecting the quality of the end product.

4. Enzyme activity may be inhibited if the volume of radioactive label present in the reaction exceeds 40% of the total reaction volume. To avoid this problem, reactions can be scaled up accordingly.

5. In the presence of Co^{2+} (present in 5X buffer), double-stranded DNA may also be labeled. DNA with a protruding 3´ terminus is preferred. Blunt-ended or recessed 3´ termini may also be labeled, although not with uniform efficiency.

IV. Determination of Percent Incorporation/Specific Activity

A. Protocol

Materials to Be Supplied by the User

(Solution compositions are provided in Section V.)

- 0.2M EDTA
- 0.5M Na_2HPO_4 (pH 6.8)
- Whatman® DE-81 2.3cm circular filters

1. Dilute 1µl of the reaction mixture into 100µl of 0.2M EDTA. Spot 3µl of this solution onto each of four Whatman® DE-81 2.3cm circular filters.
2. Dry the filters briefly under a heat lamp. Set two filters aside for use in determining total cpm.
3. Wash the other two filters in 50ml of 0.5M Na_2HPO_4 (pH 6.8) twice for 5 minutes each to remove unincorporated nucleotides.
4. Dry the washed filters under a heat lamp.
5. Add the appropriate scintillation fluid to each filter and count in a scintillation counter.

B. Example of a Standard Calculation

$$\text{\% incorporation} = \frac{\text{incorporated cpm}}{\text{total cpm}} \times 100$$

$$\text{Total cpm incorporated} = \text{incorporated cpm} \times \text{dilution factor*} \times \frac{\text{total reaction volume}}{\text{volume counted}}$$

(*Dilution factor is from Section IV.A, Step 1.)

Average number of bases added to each primer

$$= \frac{\text{\% incorporation}}{100} \times \text{molar ratio of nucleotide to primer present in the reaction}$$

Amount of DNA synthesized

= average number of bases added per primer × 330pg/pmol base × pmol primer present in reaction

$$\text{Specific activity of probe} = \frac{\text{total cpm incorporated}}{\text{µg of DNA template + µg DNA synthesized}}$$

V. Composition of Buffers and Solutions

0.2M EDTA (pH 8.0)

37.22g disodium ethylene-diaminetetraacetate •$2H_2O$

Add the EDTA to 300ml of water, adjust the pH to 8.0 with NaOH pellets and stir until the EDTA is in solution. Adjust the final volume to 500ml with water and filter sterilize.

0.5M Na_2HPO_4 (pH 6.8)

47.25g NaH_2PO_4 (monobasic)
22.35g Na_2HPO_4 (dibasic)

Add water, slowly, to a final volume of 1 liter and filter sterilize.

VI. References

1. Kato, K. *et al.* (1967) Deoxynucleotide polymerizing enzymes of calf thymus gland. II. Properties of terminal deoxynucleotidyl transferase. *J. Biol. Chem.* **242**, 2780–9.
2. Eun, H-M. (1996) *Enzymology Primer for Recombinant DNA Technology*, Academic Press, Inc., San Diego, CA.

Part# 9PIM187
Printed in USA. Revised 9/13

ambion
by life technologies

DNase I (RNase free)

Catalog Number AM2222, AM2224

Pub. No. 4393898 **Rev.** B

Contents	Quantity	Storage conditions
DNase I , 2 U/µL	AM2222: 2000 Units AM2224: 5 X 2000 Units	Store at –20°C. *Do not store in a frost-free freezer.*
10X DNase I Buffer • 100 mM Tris, pH 7.5 • 25 mM $MgCl_2$ • 5 mM $CaCl_2$	1 mL	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

DNase I (RNase-free) is a high-purity DNase I for degradation of DNA in applications where the absence of RNase is critical.

Source: Bovine pancreas

Unit (U) definition: One unit is the amount of enzyme required to completely degrade 1 µg DNA in 10 min at 37°C, and it is equivalent to 0.04 Kunitz units.

Storage buffer (*not included*): 20 mM HEPES pH 7.5, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 1 mM DTT and 50% (v/v) glycerol.

General information

DNase I (E.C. 3.1.21.1) from bovine pancreas is a 31-kDa glycoprotein. It is purified to be free of RNases and other contaminating proteins by an extensive series of chromatographic procedures. DNase I is an endonuclease that hydrolyzes phosphodiester linkages yielding oligonucleotides with a 5'-phosphate and a 3'-hydroxyl group (Kunitz, 1950). DNase I has been shown to act on single- and double-stranded DNA, chromatin, and RNA:DNA hybrids. DNase I requires bivalent cations (Mg^{2+} and Ca^{2+}) for maximal activity (Clark and Eichhorn, 1974; Junowicz and Spencer, 1973; Price, 1975).

Using DNase I (RNase-free)

DNase I (RNase-free) is used to degrade DNA in the presence of RNA when the absence of RNase is critical to maintain the integrity of the RNA. DNase Iis frequently used to remove template DNA from in vitro transcription reactions (Krieg et al, 1985), or to destroy genomic DNA in RNA preparations prior to reverse transcription-PCR (RT-PCR).

Removal of contaminating genomic DNA from RNA samples

1. If the nucleic acid solution concentration is >200 µg/mL, dilute it to 10 µg nucleic acid/50 µL.
2. Add 10X DNase I Buffer (supplied) to 1X concentration in the RNA sample.
3. Add 1 µL DNase I (2 U) for up to 10 µg RNA in a 50 µL reaction, and incubate at 37°C for 30 minutes.

   These reaction conditions will remove up to 2 µg of genomic DNA.
4. Extract the RNA sample with phenol/chloroform to inactivate the DNase I.

Degradation of DNA template in a transcription reaction

1. After transcription, add 2 U of DNase I to a 20 µL transcription reaction. It is not necessary to add 10X DNase I Buffer to the transcription reaction.
2. Incubate at 37°C for 15 minutes.
   - If the transcript is to be gel purified, then gel loading buffer may be added directly to the DNase I-treated transcription reaction.
   - If not, the DNase I can be inactivated by phenol-chloroform extraction.

Conditions for complete DNA digestion

1. Add 10X DNase I Buffer to 1X concentration in the solution to be DNase-treated, and add approximately 1–2 U of DNase I per 1 µg DNA present.

2. Incubate at 37°C for 15–30 minutes.

Heat inactivation of DNase I (RNase-free)

Some protocols suggest heating at 75°C for 5 minutes to inactivate DNase I (Huang, Fasco, and Kaminsky, 1996). We recommend a 10-minute incubation at 75°C for complete inactivation of DNase I (RNase-free) at a concentration of 0.1 U/µL. If this is the preferred method of inactivation, add EDTA to a final concentration of 5 mM before heating. If EDTA is not added, the RNA will undergo chemical scission when heated.

Note: For RNA samples that are to be used in reverse transcription reactions, the EDTA concentration in the RNA sample must be taken into account. Excess EDTA in an RNA sample may lower the free Mg^{2+} concentration and affect the efficiency of reverse transcription. After heat inactivation of DNase I. It may be necessary to add additional Mg^{2+} for maximum reverse transcriptase activity. Alternatively, DNase I can be inactivated and removed by phenol/chloroform extraction.

Gel analysis

Gel loading buffers should contain EDTA to eliminate DNase I activity; we recommend using denaturing gel loading buffers such as Gel Loading Buffer II (Cat. no. AM8546G, AM8547).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

References

Clark, R. and Eichhorn, GL. (1974) *Biochem* 13, 5098.

Huang Z, Fasco MJ, and Kaminsky LS (1996) *BioTechniques* 20,1012–1020.

Junowicz, E. and Spencer, JH. (1973) *BBA* 312, 72.

Krieg, PA, et al. (1985) *Genetic Engineering Principles and Methods* (Setlow JK, Hollaender A, ed.) Vol. 7, Plenum Press, New York, London.

Kunitz, M. (1950) *J Gen Physiol* 33, 349.

Price, P.A. (1975) *J Biol Chem* 250,1981–1986.