Product Contents

Tth DNA Polymerase:

Part No.	Size (units)
M210A	100
M210B	500

Description: *Tth* DNA Polymerase is a thermostable enzyme that replicates DNA at 74°C and exhibits a half-life of 20 minutes at 95°C (1). *Tth* catalyzes the polymerization of nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium and the polymerization of nucleotides into DNA using an RNA template in the 5´→3´ direction in the presence of manganese. The enzyme has a molecular weight of 94,000 daltons as estimated from the predicted amino acid sequence and exhibits 5´→3´ exonuclease activity. *Tth* is recommended for use in PCR, RT-PCR, reverse transcription and primer extension reactions at elevated temperature.

RT 10X Buffer (M213A): When the RT 10X Buffer supplied with this enzyme is diluted 1:10, it has a composition of 10mM Tris-HCl (pH 8.3 at 25°C) and 90mM KCl.

Thermophilic DNA Polymerase 10X Reaction Buffer, Magnesium Free (M190A): When the Thermophilic DNA Polymerase 10X Buffer supplied with this enzyme is diluted 1:10, it has a composition of 50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C) and 0.1% Triton® X-100.

Chelate 10X Buffer (M214A): When the Chelate 10X Buffer supplied with this enzyme is diluted 1:10, it has a composition of 10mM Tris-HCl (pH 8.3 at 25°C), 100mM KCl, 0.75mM EGTA, 0.05% Tween® 20 and 5% glycerol.

$MnCl_2$ Solution, 10mM (M212A): *Tth* DNA Polymerase is supplied with a 10mM solution of manganese chloride.

$MgCl_2$ Solution, 25mM (A351B): *Tth* DNA Polymerase is supplied with a 25mM solution of magnesium chloride.

Usage Note: It is important to vortex the $MnCl_2$ Solution, 10mM and the $MgCl_2$ Solution, 25mM thoroughly after thawing and prior to use to ensure homogeneity of the salts in the solutions.

Enzyme Storage Buffer: *Tth* DNA Polymerase is supplied in 10mM Tris-HCl (pH 7.5 at 25°C), 300mM KCl, 1mM DTT, 0.1mM EDTA, 0.5mg/ml BSA and 50% glycerol.

Source: Purified from *Thermus thermophilus* HB-8 (1).

Unit Definition: One unit is defined as the amount of enzyme that incorporates 10nmol of total nucleotide into TCA-precipitable form in 30 minutes at 70°C. The reaction conditions are: 50mM Tris-HCl (pH 9.0 at 25°C), 50mM NaCl, 10mM $MgCl_2$, 200µM each dNTP and activated calf thymus DNA as a substrate. See the unit concentration on the Product Information Label.

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

Quality Control Assays

Activity Assay

Functional Assay: *Tth* DNA Polymerase is tested for performance in RT-PCR. The resulting PCR product is visualized as a single band on an ethidium bromide-stained gel.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 5 units of *Tth* DNA Polymerase in Thermophilic DNA Polymerase 1X Buffer for 16 hours at 70°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting. *Tth* DNA Polymerase is also tested on lambda DNA.

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 5 units of *Tth* DNA Polymerase in Thermophilic DNA Polymerase 1X Buffer and 1mM $MnCl_2$ for RNase activity for one hour at 37°C and in Thermophilic DNA Polymerase 0.2X Buffer, Chelate 1X Buffer, 2mM $MgCl_2$ and 0.2mM $MnCl_2$ for DNase activity for one hour at 70°C. The release of radiolabeled nucleotides is monitored by scintillation counting of TCA-precipitable and TCA-soluble material. Passing specification is <3% release for RNase and <1% release for DNase.

Physical Purity: The purity is >95% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Reference

1. Ruttimann, C. *et al.* (1985) DNA polymerases from the extremely thermophilic bacterium *Thermus thermophilus* HB-8. *Eur. J. Biochem.* **149**, 41–6.

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Part# 9PIM210

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Usage Information

I. Description

Tth DNA Polymerase is a thermostable enzyme of 94kDa isolated from *Thermus thermophilus* HB-8 (1). *Tth* DNA Polymerase catalyzes the DNA-dependent polymerization of nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium chloride. The enzyme also catalyzes the polymerization of DNA using an RNA template in the presence of manganese chloride (1,2). The ability of *Tth* DNA Polymerase to reverse transcribe at elevated temperatures minimizes the problems encountered with strong secondary structures in RNA since they are unstable at higher reaction temperatures. Higher temperatures also result in increased specificity of primer hybridization and extension.

Applications of *Tth* DNA Polymerase include:

- Primer extension.
- Reverse transcription.
- cDNA synthesis.

II. Standard Applications

Reagents to Be Supplied by the User

(Solution compositions are provided in Section III.)

- [α-^{32}P]dCTP (>400Ci/mmol; optional)
- dNTP mix (Cat.# U1240 or U1330)
- downstream primer
- upstream primer
- Nuclease-Free Water (Cat.# P1193)
- EGTA, 0.5M (optional)

A. First-Strand Synthesis of cDNA

Successful cDNA synthesis is dependent on the integrity of the mRNA used as the template. Procedures for creating an RNase-free laboratory have been described (3). We also recommend the use of Recombinant RNasin® Ribonuclease Inhibitor for the protection of mRNA samples.

First-strand cDNA synthesis relies on the RNA-dependent DNA polymerase activity of *Tth* DNA Polymerase in the presence of manganese. The following procedure requires 5pmol (2 x 2.5pmol) of starting mRNA for first-strand synthesis. Total RNA (1–1,000ng) may be used as template. The amount of total RNA to use is greatly dependent upon the relative abundance of the target message. Since reaction conditions are template-dependent, the following conditions are provided as a starting point. Perform pilot reactions to optimize conditions for each template.

1. Optimize the primer:template ratio for each template. In a sterile, RNase-free microcentrifuge tube, combine the following components:

Component	Amount
RT 10X Buffer	2µl
$MnCl_2$, 10mM Solution	2µl
dNTP mix, 2mM each	2µl
[α-^{32}P]dCTP (>400Ci/mmol)	2–5µCi
downstream primer	*15pmol
Tth DNA Polymerase	4–6 units
mRNA template	2.5pmol
Nuclease-Free Water to final volume	20µl

Note: Since the entire 20µl first-strand reaction must be used for second-strand synthesis, prepare a duplicate reaction for quantitating first-strand synthesis by TCA precipitation or alkaline agarose gel analysis (4).

2. Mix gently by flicking the tube and incubate at 70°C for 20 minutes.

Note: Oligo(dT) or random hexamer primers **will not anneal** to the RNA template at 70°C. Therefore, we recommend the use of gene-specific primers designed with a melting temperature high enough to anneal at 70°C.

3. If second-strand synthesis is to be performed, place the first-strand reaction on ice and proceed to Section II.B. Otherwise, add EGTA to a final concentration of 20mM to terminate the reaction and place the sample on ice.

4. First-strand synthesis can be quantitated by measuring the incorporation of [α-^{32}P]dCTP by TCA precipitation or by alkaline agarose gel analysis (4). Since the entire 20µl first-strand reaction must be used for second-strand synthesis, a duplicate reaction is required for quantitating first-strand synthesis.

*A general formula for calculating the number of nanograms of primer equivalent to 15pmol is: 15pmol = 4.9ng x *b*, where *b* is the number of bases in the primer.

B. Second-Strand Synthesis of cDNA

Second-strand cDNA synthesis relies on the DNA-dependent DNA polymerase activity of *Tth* DNA Polymerase, which requires the removal of manganese and the addition of magnesium. Removal of manganese is accomplished by chelation with EGTA, which is present in the Chelate 10X Buffer.

1. Prepare the following second-strand reaction mix:

Component	Amount
upstream primer	*15pmol
Chelate 10X Buffer	8µl
$MgCl_2$, 25mM Solution	8µl
Nuclease-Free Water to final volume	80µl

2. Centrifuge the first-strand reaction from Section II.A in a microcentrifuge for 5 seconds to collect any condensate.
3. Add 80µl of the second-strand reaction mix to 20µl of the first-strand reaction. Mix gently by flicking the tube. Heat at 95°C for 5 minutes to denature the RNA:DNA hybrids.
4. Incubate the reactions at 70°C for 20 minutes. Alternatively, second-strand synthesis can be accomplished by a cycled reaction.
5. The results of the second-strand reaction can be quantitated by measuring the incorporation of [α-^{32}P]dCTP by TCA precipitation or by agarose gel analysis (4).
6. Purify the reaction products using the Wizard® PCR Preps DNA Purification System (Cat.# A7170; reference 5).

III. Composition of Buffers and Solutions

Chelate 10X Buffer (provided)

100mM	Tris-HCl (pH 8.3)
1M	KCl
7.5mM	EGTA
0.5%	Tween® 20
50%	glycerol

dNTP mix

2mM each of dATP, dCTP, dGTP and dTTP in water

Bulk dNTPs are available from Promega; Cat.# U1240 or U1330.

EGTA, 0.5M

Dissolve 19.2g of EGTA in 70ml of deionized water, adjust the pH to 8.0 with 10N NaOH and add deionized water to 100ml final volume. Filter-sterilize (0.22µm) and store at room temperature.

RT 10X Buffer (provided)

100mM	Tris-HCl (pH 8.3 at 25°C)
900mM	KCl

Thermophilic DNA Polymerase 10X Buffer (provided)

500mM	KCl
100mM	Tris-HCl (pH 9.0 at 25°C)
1%	Triton® X-100

Buffer is optimized for use with the dNTP mix (0.2mM each dNTP).

IV. References

1. Rüttimann, C. *et al.* (1985) *Eur. J. Biochem.* **149,** 41–6.
2. Myers, T.W. and Gelfand, D.H. (1991) *Biochemistry* **30,** 7661–6.
3. Blumberg, D.D. (1987) *Meth. Enzymol.* **152,** 20–4.
4. *Protocols and Applications Guide,* Third Edition (1996) Promega Corporation.
5. *Wizard® PCR Preps DNA Purification System Technical Bulletin* #TB118, Promega Corporation.

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