**Tth DNA Polymerase**

from *Thermus thermophilus* strain HB8

**Product No. A5190**

**Description**

**Introduction**

*Tth* DNA Polymerase is a thermostable enzyme of approximately 94 kDa, isolated from the eubacterium *Thermus thermophilus* strain HB8. This enzyme replicates DNA at 74°C and reveals RNA-dependent DNA-polymerase activity in the presence of Mn²⁺ ions. Meanwhile, the concentration of RNA template for effective reverse transcription with *Tth* DNA polymerase should be higher as compared to reverse transcription directed by Reverse Transcriptases (M-MuLV, AMV).

*Tth* DNA Polymerase is a thermostable DNA polymerase with intrinsic reverse transcription (RT) activity, but no RNase H activity (6.). The error rate of *Tth* DNA polymerase increases in the presence of Mn²⁺ ions (Ref. 5, 11, 12). *Tth* DNA polymerase can reverse transcribe and amplify fragments up to 2 - 3 kb. However, the fragment should be ideally smaller than 1 kb. The error rate is 3.0 x 10⁻⁵ in PCR reactions. Although *Tth* DNA polymerase adds 3’dA overhangs, it is not recommended for PCR product cloning because the error rate is similar to *Taq* polymerase. *Tth* DNA polymerase accepts modified dNTPs and can therefore be used to label DNA fragments with modified dNTPs, e.g. labeled with digoxigenin, biotin or fluorescein.

Several buffer systems have been used for 1-tube or step (6.) and 2-tubes/steps RT-PCR (5) with *Tth* DNA Polymerase. One step means intrinsic reverse transcription and amplification is mediated by one enzyme in one tube. Two steps means that the first step is a RT-PCR reaction (first strand synthesis of the cDNA) with the reverse primer and a reverse transcription buffer including Mn²⁺, followed by a second PCR reaction (including the forward primer, second strand synthesis of cDNA) with a PCR buffer (Mg²⁺).

*Tth* DNA Polymerase displays the unique property of maintaining both DNA- and RNA-dependent DNA polymerase activities in the presence of 2 - 5 % (v/v) of phenol-saturated PBS buffer. *Tth* DNA polymerase mediated reverse transcriptase activity was unaffected by phenol-saturated phosphate-buffered saline concentrations as high as 15 % (v/v). By contrast, *Taq* DNA Polymerase was inactive under these conditions. The ability to work in the presence of phenol can greatly simplify reverse transcription, PCR and reverse transcription-PCR protocols since the phenol-saturated aqueous phase of a phenol partition can be added directly to the reaction mixtures (8).

**Unit definition:** One unit of activity is the amount of enzyme required to incorporate 10 nmoles of dNTP into an acid-insoluble DNA fraction in 30 minutes at 70°C.

**Supplied in Storage buffer:**
10 Tris • HCl (pH 7.5), 300 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50 % glycerol

**Supplied with Reaction buffers:**
- **(A)** RT-PCR buffer, one-step reaction (5X)
  250 mM bicine/KOH (pH 8.2, 25°C), 575 mM KOAc, 40 % glycerol (v/v)
- **(B)** Reverse transcription buffer (10X)
  100 mM Tris • HCl (pH 8.9, 25°C), 900 mM KCl
- **(C)** Tth PCR buffer (10X)
  100 mM Tris • HCl (pH 8.9, 25°C), 1 M KCl, 500 µg/ml BSA, 0.5 % Tween® 20, 15 mM MgCl₂
- **(D)** MnCl₂ (1 ml)
  100 mM
- **(E)** MgCl₂ (1 ml)
  100 mM

**Recommended Mg and Mn concentration:**
- 3 - 6 mM Mg²⁺
- 1 - 2 mM Mn²⁺ for RNA-dependent cDNA synthesis.

**Storage Conditions:**
-20°C

**Concentration:**
5000 units/ml
RNA Preparation

Successful RT-PCR depends on the quality of the RNA. Use highest purity of RNA (A_{260}/A_{280} ratio of 1.7 or higher). RNA should be DNA-free. Total RNA, messenger RNA or viral RNA can be used, too. The quality of template RNA can be assessed using a positive control primer pair for a housekeeping gene (β-actin, GAPDH). The 16S rRNA gene from various bacterial cultures was amplified by the polymerase chain reaction without DNA purification, and sequenced directly by using a laser fluorescence DNA sequencer and Tth polymerase with a cycle sequencing protocol. The described procedures provides almost complete 16S rDNA sequence data within a couple of days and facilitate systematic studies (9).

A. Reverse transcription polymerase chain reaction (RT-PCR):

Since Tth-DNA polymerase shows reverse transcriptase activity and is active in the presence of manganese and accepts both RNA and DNA as matrix, the whole reaction can be performed as a “one-step” RT-PCR analysis (6). This experimental approach includes the amplification of fragments up to a maximum size of 1 kb and a relatively high error rate for DNA polymerase activity as a result of the manganese ion concentration (7). Reverse transcription can be performed at 60°C, minimizing problems arising from the RNA secondary structure and a high G/C content.

1. One step RT-PCR:

The “one enzyme/one tube” Tth DNA polymerase assay uses bicine buffers containing Mn^{2+} ions, compatible with both RT and subsequent PCR (6, 10). For RT-PCR amplification (reverse transcription and amplification in one step), the concentration of Mn^{2+} has to be adjusted to 1 - 4 mM for each reaction. The one step reaction eliminates the risk of cross contaminations associated with the two step RT-PCR.

Example:

RT-PCR buffer, one-step reaction (5x): 250 mM bicine/KOH (pH 8.2, 25°C); 575 mM KOAc, 40 % glycerol (v/v).
Mn^{2+} ions (100 mM solution is supplied) : 2.5 mM (test 1 - 4 mM)
5 units Tth DNA polymerase
Template up to 1 µg (dilute 1:10 and analyze 1 ng, 10 ng, 100 ng and 1000 ng of your template RNA)
Primers (forward and reverse): 450 nm
dNTPs 200 - 300 µM

Thermocycles:

The number of cycles ranges from 20 to 50 in the literature. If the template is limited, increased number of cycles may result in nonspecific product yield.

Example:

1 × RT-reaction at 60 - 70°C *, 30 minutes
1 × initial denaturation at 94°C, 1 - 2 minutes
10 × denaturation at 94°C, 30 s – 1 minute
annealing at 50 - 70°C*, 30 - 90 seconds
elongation at 60 - 70°C, 45 - 90 seconds
20-30 × denaturation at 94°C, 30 seconds
annealing at 50 - 70°C*, 30 seconds
elongation at 60 - 70°C, 45 seconds
1 × final elongation time at 72°C for 7 minutes
analyze on 1 - 2 % agarose gel.

*depends on your primers, 70 - 75°C is the optimal reaction temperature for Tth DNA polymerase. Tth DNA polymerase is resistant to prolonged incubations (20 minutes half life at 95°C) at high temperatures and can therefore be used for PCR.
2. Two step RT-PCR:

Two steps means that the first step is a RT-PCR reaction (first strand synthesis of the cDNA) with the reverse primer and a reverse transcription buffer, including Mn²⁺, followed by a second PCR reaction (including the forward primer, second strand synthesis of cDNA) with a PCR buffer (Mg²⁺). The error rate of Tth DNA polymerase is increased in the presence of Mn²⁺ ions (5). Therefore, a two step RT-PCR is recommended for PCR products that have to be cloned and used for subsequent investigations.

1. Step: Reverse transcription reaction:
Prepare reaction on ice.
10X Reverse transcription buffer: 100 mM Tris · HCl (pH 8.9, 25°C), 900 mM KCl
100 mM MnCl₂ (supplied) : 1 - 2 mM final concentration
dNTPs: 200 µM final each
reverse primer : 750 nM
template RNA: 200 ng
Tth DNA polymerase: 4 - 5 units
**Add sterile H₂O up to 20 µl and incubate at 60 - 70°C * for 30 minutes**
*depends on your primers, 70 - 75°C is the optimal reaction temperature for Tth DNA polymerase.

2. Step: PCR reaction
Add to the reverse transcription reaction (20 µl) a PCR master mix with a volume of 80 µl, resulting in a final volume of 100 µl. Prepare reaction at room temperature.
add 0.8 µl 10X PCR buffer: 100 mM Tris · HCl (pH 8.9, 25°C), 1 M KCl, 500 µg/ml BSA, 0.5 % Tween® 20, 15 mM MgCl₂
add 10 µl of a 7.5 mM EGTA** solution (0.75 mM EGTA final)
forward primer: 750 nM
Add sterile H₂O up to 80 µl
Mix (20 µl from a. after the incubation with 80 µl from b.) and centrifuge.
Place the 100 µl sample on thermocycler:
1 × initial denaturation at 94°C, 2 minutes
10 × Denaturation at 94°C, 30 seconds - 1 minute
annealing at 50 - 70°C*, 30 - 90 seconds
elongation at 60 - 70°C, 45 - 90 seconds
20 - 30 × denaturation at 94°C, 30 seconds
annealing at 50 - 70°C*, 30 seconds
elongation at 60 - 70°C, 45 seconds
1 × finale elongation time at 60 - 70°C for 7 minutes
*depends on your primers, 70 - 75°C is the optimal reaction temperature for Tth DNA polymerase.
analyze on 1 - 2 % agarose gel.
B. Standard PCR protocol

**PCR reaction:**
The Mg$^{2+}$ concentration as well as the enzyme concentration have to be optimized for PCR amplification. The typical range of the Mg$^{2+}$ concentration is 1 - 6 mM; the standard concentration is 1.5 mM.

Optimal enzyme concentration is 0.5 - 5.0 units; the standard concentration is 2.5 units

Template: up to 1 µg (dilute 1 : 10 and analyze 1 ng, 10 ng, 100 ng and 1000 ng of your template RNA).

**For example:**
10X Tth DNA polymerase PCR buffer: 100 mM Tris · HCl (pH 8.9, 25°C), 1 M KCl, 500 µg/ml BSA, 0.5% Tween 20 and 15 mM MgCl$_2$

dNTPs: 200 µM

primers (forward and reverse): each 400 nM

template DNA: up to 0.5 µg

Tth DNA polymerase: 2.5 units

Add sterile H$_2$O up to x µl

**Thermocycler:**
1 × initial denaturation at 94°C, 2 minutes

10 × denaturation at 94°C, 30 seconds - 1 minutes

annealing at 50 - 70°C*, 30 - 90 seconds

elongation at 72°C, 45 - 90 seconds

20 × denaturation at 94°C, 30 seconds

annealing at 50 - 70°C*, 30 seconds

elongation at 72°C, 45 seconds

1 × finale extension at 72°C for 7 minutes.

analyze on 1 - 2% agarose gel.

** For a 0.5 M EGTA stock: dissolve 19.2 g EGTA in 70 ml deionized water, adjust pH 8.0 with NaOH (10 M). Add deionized water to 100 ml final volume. Filter sterilize with 0.22 µm and store at room temperature.

Literature
(11) Fromant et al. () Anal. Biochem. 224, 347-353
(12) Beckman et al. () Biochemistry 24, 5810-5817