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Tth DNA Polymerase

Description *Tth* DNA Polymerase is a thermostable enzyme of approximately 94kDa isolated from eubacterium *Thermus thermophilus* strain HB8. This enzyme replicates DNA at 74℃, reveals RNA-dependent DNA-polymerase activity in the presence of Mn^{2+} ions. Meanwhile, the concentration of RNA template for effective reverse transcription with Tth DNA polymerase should be higher if to compare with reverse transcription directed by Reverse Transcriptases (M-MuLV, AMV).

Concentration 5000units/ml

Storage buffer 10 TrisHCl, pH 7.5, 300mM KCl, 0.1 mM EDTA, 1mM DTT, 50% glycerol.

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

Reaction buffers:
1: RT-PCR buffer, one-step reaction (5 x):
250 mM bicine/KOH, pH 8.2 (25° C); 575 mM K-acetate, 40% glycerol (v/v)

2: 10 x Reverse transcription buffer:
100 mM Tris-HCl (pH 8.9 at 25° C), 900 mM KCl

3: 10 x *Tth* PCR buffer:
100 mM Tris-HCl (pH 8.9 at 25° C), 1M KCl, 500 µg/ml BSA, 0.5% Tween 20 and 15 mM $MgCl_2$

Supplied with one tube $MnCl_2$ (100mM, 1ml) and one tube $MgCl_2$ (100mM, 1ml)

Recommended concentration of Mg^{2+} - 3 – 6 mM**,** Mn^{2+}- 1-2 mM for RNA-dependent cDNA synthesis.

Storage conditions -20°C

Description and Standard-protocol

Tth DNA Polymerase is a thermostable DNA polymerase with intrinsic reverse transcription (RT), but no RNAse H activity (6.). The error rate of *Tth* DNA polymerase increase in the present of Mn^{2+} ions (5., 8., 12 and 13.).*Tth* DNA polymerase can reverse transcript and amplify fragments up to 2-3 kb. However, the fragment should be ideally smaller 1 kb. The error rate is 3.0×10^{-5} in PCR reaction(8.). 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 labeled with digoxigenin, biotin or fluorescein. Several buffer systems were used for 1-tube or step (6.) and 2-tubes/steps RT-PCR (5.) with *Tth*-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 which is including Mn^{2+} which is followed by a second PCR reaction (including the forward primer, second strand synthesis of cDNA) with a PCR buffer (Mg^{2+}).
Tth DNA polymerase displayed the unique property of maintaining both DNA- and RNA-dependent DNA polymerase activities in the presence of 2%-5% (vol/vol) 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% (vol/vol). By contrast, Taq DNA Polymerase was inactive under these conditions. The ability to function in the presence of phenol can greatly simplify reverse transcriptase, PCR and reverse transcription-PCR protocols since the phenol-saturated aqueous phase of a phenol partition can be added directly to the reaction mixtures (9.).

Generally:

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. The quality of template RNA can be assessed using a positive control primer pair for a housekeeping gene (ß-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 fluorescent DNA sequencer and *Tth* polymerase with a cycle sequencing protocol. The described procedures provide almost complete 16S rDNA sequence data within a couple of days and facilitate systematic studies (10.).

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

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The use of *Tth*-DNA polymerase which, is also a reverse transcriptase, is active in the presence of manganese and accepts both RNA and DNA as matrix, enables the whole reaction to be performed as a “one-step” RT-PCR analysis (6). This experimental approach include the amplification of fragments only to a maximum of 1 kb and a relatively high error rate for DNA polymerase as a result of the manganese ion concentration (7.).

Reverse transcription could be performed at 60° C minimizing problems from RNA secondary structure and 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 that are compatible with both RT and subsequent PCR (6., 11.)

For RT-PCR amplification (reverse transcription and amplification in one step), the

concentration of Mn^{2+} needs to be determined by testing Mn^{2+}-concentrations from 1- 4 mM for each reaction.

One step reaction eliminates the risk of cross contaminations associated with two step RT-PCR.

For example:

RT-PCR buffer, one-step reaction (5 x): 250 mM bicine/KOH, pH 8.2 (25° C); 575 mM K-acetate, 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 1ng, 10 ng, 100 ng and 1000 ng of your template RNA)

Primers (forward and reverse): 450 nm

dNTPs: 200-300 µM

Thermocycles:

Number of cycles ranges from 20 – 50 in the literature. If the template is limited, increasing the number of cycles may result in nonspecific product yield.

For example:

1 × RT-reaction at 60°- 70° C *, 30 min

1 × initial denaturation at 94° C, 1 – 2 min

10 × denaturation at 94° C, 30 s – 1 min

annealing at 50°-70° C*, 30 - 90 s

elongation at 60-70° C, 45 – 90 s

20-30 × denaturation at 94° C, 30 s

annealing at 50°-70° C*, 30 s

elongation at 60-70° C, 45 s

1 × final elongation time at 72° C for 7 min.

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 min Half - Life time at 95° C) at high temperatures (94° C) and can therefore be used for PCR.

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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 which is including Mn^{2+} which is followed by a second PCR reaction (including the forward primer, second strand synthesis of cDNA) with a PCR buffer (Mg^{2+}).
The error rate of *Tth* DNA polymerase is increased in present of Mn^{2+} – ions (5). Therefore a Two step RT – PCR is recommended for PCr products which are cloned and used for subsequent further investigations.

2. reverse transcription reaction :

Prepare reaction on ice.

10 x Reverse transcription buffer: 100 mM Tris-HCl (pH 8.9 at 25° C), 900 mM KCl

100 mM $MnCl_2$ (supplied) : 1 – 2 mM final concentration

dNTPs: each 200 µM final

reverse primer : 750 nM

template RNA: 200 ng

Tth DNA polymerase: 4 –5 units

Add sterile H_2O up to 20 µl and incubate at 60°- 70° C *, 30 min

*depends on your primers, 70 - 75° C is the optimal reaction temperature for *Tth* DNA polymerase.

2. PCR reaction

Add to the reverse transcription reaction (20 µl) a PCR master mix with a volume of 80 µl, so that the final volume is 100 µl. Prepare reaction at room temperature.

10 x PCR buffer: 100 mM Tris-HCl (pH 8.9 at 25° C), 1M KCl, 500 µg/ml BSA, 0.5% Tween 20 and 15 mM $MgCl_2$
add 0.8 µl
0.75 mM EGTA final, add 10 µl of a 7.5 mM EGTA** solution.
forward primer : 750 nM
Add sterile H_2O up to 80 µl
Mix (20µl 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 min

10 × Denaturation at 94° C, 30 s – 1 min

annealing at 50°-70° C*, 30 – 90s

elongation at 60 - 70° C, 45 - 90 s

20-30 × denaturation at 94° C, 30 s

annealing at 50°-70° C*, 30 s

elongation at 60 - 70° C, 45 s,

1 × finale elongation time at 60 - 70° C for 7 min

*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

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PCR reaction:

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

Optimal enzyme concentration: 0.5 – 5.0 units; the standard concentration is 2.5 units

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

For example:

10 x *Tth* DNA polymerase PCR buffer: 100 mM Tris-HCl (pH 8.9 at 25° C), 1M 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_2O up to x µl

Thermocycler:

1 × initiale denaturation at 94° C, 2 min

10 × denaturation at 94° C, 30 s – 1min

annealing at 50°-70° C*, 30 – 90 s

elongation at 72° C, 45 - 90 s

20 × denaturation at 94° C, 30 s

annealing at 50°-70° C*, 30 s

elongation at 72° C, 45 s

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

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).

Catalog #	Pack size
104005	500 u
104025	2500 u

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