Tth Polymerase

Description: *Tth* Polymerase is a recombinant thermostable enzyme of approximately 94 kDa isolated from eubacterium *Thermus thermophilus* strain HB8 and expressed in bacterium *E.coli*. This enzyme replicates DNA at 74 °C. It shows RNA-dependent DNA-polymerase activity in the presence of Mn^{2+} ions. Tth Polymerase has a intrinsic **reverse transcription (RT)**, but no RNAse H activity. Tth DNA polymerase can reverse transcript and amplify fragments up to 2 - 3 kb.

Content

Ref No.	S104005	104005	104025	color
***Tth* Polymerase**	**Sample size**	**500 units**	**2500 units**	**blue**
***Tth* PCR buffer (10x) ***	**1 mL**	**1 mL**	**5x 1 mL**	**red**
RT-PCR buffer **, one step reaction (5x)	**1 mL**	**1 mL**	**5x 1 mL**	**black**
Reverse Transcription buffer (10x) ***	**1 mL**	**1 mL**	**5x 1 mL**	**yellow**
$MnCl_2$ 100 mM	**1 mL**	**1 mL**	**5x 1 mL**	**white**
Datasheet	**1**	**1**	**1**	**--**

* 100 mM Tris-HCl (pH 8.9 at 25° C), 1M KCl, 500 µg/mL BSA, 0.5 % Tween 20 and 15 mM $MgCl_2$

** 250 mM bicine/KOH, pH 8.2 (25° C); 575 mM K-acetate, 40% glycerol (v/v).

*** 100 mM Tris-HCl (pH 8.9 at 25° C), 900 mM KCl.

Applications: *Tth* Polymerase is used for RT - PCR. Although *Tth* Polymerase adds 3´dA overhangs, it is not recommended for PCR product cloning because the error rate (3.0×10^{-5}) is similar to *Taq* DNA polymerase.
Tth Polymerase accepts modified dNTPs and can therefore be used to label DNA fragments with modified dNTPs labeled with digoxigenin, biotin or fluorescein.

Concentration: 5 units/µL

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.

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

Quality Control

- 98 % protein homogeneity in 10 % SDS-PAGE
- First strand cDNA synthesis
- No detectable exo-/endonuclease and RNase activities
- PCR amplification tests with cDNA templates

Storage conditions: -20 ˚C

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Thermocycler protocol

A Reverse transcription polymerase chain reaction (RT-PCR)

One step RT – PCR

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

Mixture preparation

Components	Volume	Final concentration
dNTP-Mix (Ref No. 110001)	x µL	200 - 300 µM
Forward Primer	variable	450 µM
Reverse Primer	variable	450 µM
Template RNA	variable	variable
5x RT-PCR buffer one step	5/10 µL	1x
$MnCl_2$	variable	1 - 4 mM
Tth Polymerase	variable	2.5 - 5 units
Sterile dest. water	Adjust to 25/ 50 µL final volume	

Prepare mix (see table above) **on ice**, vortex gently and start PCR program (see table below).

One step RT-PCR Cycle Protocol

Step	Time	Temperature
Reverse Transcription Reaction		
Initial Denaturation	1 – 2 min	94 °C
RT-Reaction	30 min	60 °C – 70 °C
PCR-Reaction (up to 40 cycles)		
Initial Denaturation	30 s – 1 min	94 °C
Denaturation	30 s – 1 min	94 °C
Annealing	30 s – 1 min	50 °C - 70 °C
Elongation	45 s – 90 s	72 °C
Final Elongation	5 min	72 °C

Two step RT – PCR

First step: RT-PCR reaction (first strand synthesis of the cDNA) with a reverse primer and a reverse transcription buffer (with Mn^{2+}).
Second step: PCR reaction (including the forward primer, second strand synthesis of cDNA) with a PCR buffer (contains Mg^{2+}).

Note: The error rate of *Tth* Polymerase is increased in present of Mn^{2+} ions. Therefore, a two-step RT – PCR is recommended for PCR products which are cloned and used for subsequent further investigations.

Setup Two-step RT-PCR reaction

Prepare reaction on ice.

First step: reaction mix for Reverse transcription reaction

Components	Volume	Final concentration
10x Reverse transcription buffer	2 µL	1x
$MnCl_2$	variable	1 - 2 mM
dNTP mix	variable	200 µM
Primer for RT	variable	450 µM
Template RNA	variable	variable
Tth polymerase	0.8 – 1 µL	4 - 5 units
Sterile dest. water	Adjust to 20 µL final volume	

Second step: reaction mix for PCR reaction

Components	Volume	Final concentration
10x PCR reaction buffer	10 µL	1x
dNTP mix	variable	200 µM
Reverse Primer	variable	450 µM
Forward Primer	variable	150 µM
Tth polymerase	0.8 – 1 µL	4 - 5 units
Sterile dest. water	Adjust to 80 µL final volume	

Gently vortex and add the 80 µL PCR reaction mix to the 20 µL reverse transcription mix after reverse transcription reaction. The total PCR volume is 100 µL.

Two step RT-PCR Cycle protocol

Step	Time	Temperature
Initial Denaturation	1 – 2 min	94 °C
Number of cycles: up to 40		
Denaturation	30 s - 1 min	94 °C
Annealing	30 s – 1 min	50 °C – 70 °C
Elongation	45 s – 90 s	72 °C
Final Elongation	5 min	72 °C

B Standard PCR protocol

Tth polymerase can be used like *Taq* polymerase for a standard PCR.

Reaction Mix for Standard PCR

Components	Volume	Final concentration
dNTP mix	variable	200 µM
Reverse Primer	variable	400 µM
Forward Primer	variable	400 µM
Template DNA	variable	0.1 - 1µg
10x PCR reaction buffer	5/10 µL	1x
dNTP mix	variable	200 µM
Tth polymerase	0.8 - 1µL	4 - 5 units
Sterile dest. water	Adjust to 25/50 µL final volume	

Combine Mix in ice and gently vortex the final mixture in a PCR tube. Place the tube into a Thermal cycler and run PCR immediately.

Standard PCR Cycle Protocol

Step	Time	Temperature
Initial Denaturation	1 – 2 min	94 °C
Number of cycles: up to 40		
Denaturation	30 s - 1 min	94 °C
Annealing	30 s – 1 min	50 °C – 70 °C
Elongation	45 s – 90 s	72 °C
Final Elongation	5 min	72 °C

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