Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Tth DNA Polymerase

Product Number: TT01

Storage

at -20°C for 24 months,transporting on blue ice

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 isolated from eubacterium Thermus thermophilus strain HB8. 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.

The Recombinant Tth enzyme with both intrinsic transcriptase and thermostable DNA polymerase activity, a convenient solution for single tube RT-PCR. The enzyme tolerates temperatures up to +50°C - in range of +50°C to +70°C for the RT reaction, and up to +95°C for the PCR, overcoming problems caused by RNA secondary structures. Carryover prevention via the incorporation of dUTP and subsequent treatment with UNG is also possible.

Features

The thermostability and the reverse transcriptase (RT) activity of Tth DNA polymerase is useful in amplifying DNA from RNA templates that contain G-C-rich sequences or secondary structures since the elevated temperatures serve to denature the template RNA. Higher temperatures (in contrast to other enzymes for RT-PCR) also result in increased specificity of primer hybridization and extension. 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 Transcriptase (M-MuLV, AMV).

Application

PCR and RT-PCR - cDNA synthesis

Concentration

5u/µl

Storage buffer

10mM Tris-HCl, 1mM dithiothreitol, 0.1 mM EDTA, 300mM KCl, 0.1% Triton X-100 (v/v), 50% glycerol (v/v), pH 7.5 (25°C).

Reaction Buffer

1. 5× RT/PCR reaction buffer (One Step-buffer): 250 mM bicine (pH 8.2, by KOH, at 25 °C), 580mM KOAC, 40% Glycerol
2. 10× PCR buffer: 100 mM Tris-HCl, (pH 8.8 at 25 °C), 15 mM MgSO4, 800mM (NH4)2SO4, 0.5 mg/ml BSA, 0.5% Tween 20

Protocol

1. One step RT PCR:Reverse transcription and amplification in one Tube.Advantage: The One step reaction eliminates the risk of cross contaminations associated with two step RT-PCR.

   1.1. One step RT-PCR Prepare two master mixes 25µl each:

Mix I:

Component	Volume	final conc.
dNTP Mix (40mM = 10mM each)	1.5 µl	300 µM

sterile Water	up to 25 µl	
forward primer	var.	450 µM
reverse primer	var.	450 µM
template RNA	var.	up to 1 µg (in steps of 1 ng, 10 ng, 100 ng, 1 µg)
Total	**25 µl**	

Mix II:

Component	Volume	final conc.
5× RT-PCR buffer	10 µl	1×
MnCl2 (25 mM)	5 µl	2.5 mM
Tth DNA Pol. Maximo	0.5 - 1 µl	2.5 - 5 units
Total	**25 µl**	

Note:1)combine Mix I and Mix II on ice and gently vortex the final mixture in a PCR-tube
2)collect the mixture from the tube and start cycling immediately

Cycling: One step RT PCR:

Step	Cycle	Time	Temperature
RT-reaction	1	30 min	60-70 °C
Initial denaturation	1	1-3 min	95 °C
10 Cycles: Denaturation Annealing [1.] Extension		30-60 sec 30-60 sec 45 sec	94-95°C; 50-70°C; 72-74 °C
20-30 cycles [3.] Denaturation Annealing [1.] Extension		30 sec 30 sec 45 sec [2.]	94-95°C; 50-70 °C; 72-74 °C
Final extension		7 min	72-74 °C

Note:1)temperature depends on the melting temp of the primer; approximately 5°C to 8°C below Tm of primers.
2)we recommend to add 5 sec per cycle extension.
3)depends on the copy number of the RNA.

2. Two step RT PCR

2.1. **Two step RT PCR (recommendation, buffer is not provided with this product)**

Component for RT-reaction	Volume	final conc.
sterile Water	up to 20 µl	
10× Reaction buffer Rev. Transcription	2 µl	1×
MnCl2	2 µl	0.9 mM
dNTP Mix (40mM = 10mM each)	0.4 µl	200 µM
reverse primer	var.	450 µM
template RNA	var.	up to 200 ng
Tth Polymerase (5µ/µl)	0.8 µl	4 units
Total for the RT reaction incubate the mixture at: 60-70 °C for 10-30 min.	20 µl	

Component for PCR-reaction	Volume	final conc.
sterile Water	up to 80 µl	
10× PCR-Reaction buffer	8 µl	0.8×
dNTP Mix (40mM = 10mM each)	0.4 µl	200 µM
reverse primer	var.	450 µM

EGTA, 7,5 mM	10 µl	0.75 nM
forward primer	var.	150 nM
Tth Polymerase (5µ/µl)	0.8 µl	4 units
Total gently vortex and add the 80 µl PCR master mix to the RT-PCR reaction (after incubation) at room temperature. Total volume:continue cycling immediately! see next line	80 µl 100 µl	

Step (PCR reaction)	Cycle	Time	Temperature
Initial denaturation	1	1-2 min	95 °C
10 Cycles Denaturation Annealing [1.)] Extension		30-60 sec 30-60 sec 45 sec	94-95°C;50-70°C;72-74°C
20-30 cycles [3.)] Denaturation Annealing [1.)] Extension		30 sec 30 sec 45 sec [2.)]	94-95°C;50-70°C;72-74°C
Final extension		7 min	72-74°C

Note:1)temperature depends on the melting temp of the primer; approximately 5 °C to 8 °C below Tm of primers.
2)we recommend to add 5 sec per cycle extension.
3)depends on the copy number of the RNA.

3. Standard PCR

Prepare two master mixes 50µl each:

Mix I:

Component	Volume	final conc.
dNTP Mix (40mM = 10mM each)	200 µl	200 µM
sterile Water	up to 50 µl	
forward primer	var.	400 µM
reverse primer	var.	400 µM
template RNA	var.	up to 0.5 µg
Total	**50 µl**	

Mix II:

Component	Volume	final conc.
sterile water	up to 50 µl	
10× PCR buffer	10 µl	1×
Tth DNA Pol. Maximo	0.5 - 0.8 µl	2.5 - 4 units
Total	**50 µl**	

Note: - combine Mix I and Mix II on ice and gently vortex the final mixture in a PCR-tube - collect the mixture from the tube and start cycling immediately.

Step (PCR reaction)	Cycle	Time	Temperature
Initial denaturation	1	1-2 min	94-95 °C
10 Cycles: Denaturation Annealing [1.)] Extension		30-60 sec 30-60 sec 45 sec	94-95°C;50-70°C;72-74 °C
20-25 cycles [3.)] Denaturation Annealing [1.)] Extension		30 sec 30 sec 45 sec [2.)]	94-95°C;50-70°C;72-74°C
Final extension		7 min	72-74°C

Note:1)temperature depends on the melting temp of the primer; approximately 5 °C to 8 °C below Tm of primers.
2)we recommend to add 5 sec per cycle extension.
3)depends on the copy number of the RNA.