

Tth DNA Polymerase

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 Transcriptases (M-MuLV, AMV).

Applications:

- PCR and RT-PCR
- cDNA synthesis

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.

Concentration: 5 u/μl

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

Reaction Buffer:

5X RT/PCR reaction buffer: 250 mM bicine (pH 8.2, by KOH, at 25 °C), 580mM KOAC, 40% Glycerol

10X 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

Usage:

1.) One step RT PCR:

- Reverse transcription and amplification in one Tube

- **Advantage:** The one step One step reaction eliminates the risk of cross contaminations associated with two step RT-PCR.

2.) Two step RT PCR

3.) Standard PCR

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.
5X RT-PCR buffer	10 µl	1X
MnCl ₂ (25 mM)	5 µl	2,5 mM
Tth DNA Pol. Maximo	0.5 - 1 µl	2.5 - 5 units
Total	25 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

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

^{1.)} temperature depends on the melting temp of the primer; approximately 5°C to 8°C below T_m 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

Component for RT-reaction	Volume	final conc.
sterile Water	up to 20 µl	
10x Reaction buffer Rev. Transcription	2 µl	1X
MnCl ₂	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 <i>for the RT reaction incubate the mixture at:</i> 60-70 °C for 10-30 min.	20 µl	

Components for PCR-reaction	Volume	final conc.
sterile Water	up to 80 µl	
10x PCR-Reaction buffer	8 µl	0.8X
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	80 µl	
<i>gently vortex and add the 80 µl PCR-mastermix to the RT-PCR reaction (after incubation) at room temperature.</i>		
Total volume:		
<i>continue cycling immediatelly! see next line</i>		
	100 µl	

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

1.) temperature depends on the melting temp of the primer; approximately 5°C to 8°C below T_m 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 50 µl 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	
10X PCR buffer	10 µl	1X
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

^{1.)} temperature depends on the melting temp of the primer; approximately 5 °C to 8 °C below T_m of primers

^{2.)} we recommend to add 5 sec per cycle extension

^{3.)} depends on the copy number of the RNA

Transportation: on blue ice

Storage: at -20 °C for 24 months

Ordering information:

Cat.-no	Description	Amount
S123	MaximoTth DNA Polymerase	250 units
S124	MaximoTth DNA Polymerase	2 x 250 units
S125	MaximoTth DNA Polymerase	10 x 250 units

[Tth DNA Polymerase Maximo: Deutsche Beschreibung](#)