

Catalog
TBP0161-500

Unit
500 U

Description

Tth DNA Polymerase is a thermostable enzyme from the eubacterium *Thermus thermophilus* strain HB8 that works best at 74 °C and can last about 20 minutes at 95 °C. It builds DNA strands in the 5'→3' direction using DNA templates (with magnesium) or RNA templates (with manganese). It is commonly used in high-temperature applications like PCR, RT-PCR, reverse transcription, and primer extension.

Specification

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:

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

Application

PCR and RT-PCR- cDNA synthesis

Protocol

- 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 25μl	
Forward primer	var.	450 μM
Reverse primer	var.	450 μM
Template RNA	var.	Up to 1μg (in steps of 1ng, 10ng, 100ng, 1μg)
Total	25μl	

Mix II:

Component	Volume	Final conc.
5 × RT-PCR buffer	10 μl	1×
MnCl ₂ (25mM)	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	30min	60-70 °C
Initial denaturation	1	1-3min	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:

- Temperature depends on the melting temperature of the primer; approximately 5°C to 8°C below the temperature of primers.
 - We recommend adding 5 seconds per cycle extension.
 - It depends on the copy number of the RNA.
- Two step RT PCR
 - Two step RT PCR (recommendation, buffer is not provided with this product)

Component for RT-reaction	Volume	Final conc.
Sterile Water	Up to 20μl	
10× Reaction buffer Rev. Transcription	2μl	1×
MnCl ₂	2μl	0.9 mM
dNTP Mix (40mM = 10mM each)	0.4μl	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 80μl	
10× PCR-Reaction buffer	8 μl	0.8×

Tth DNA Polymerase

dNTP Mix (40mM = 10mM each)	0.4 µl	200 µM
Reverse primer	var	450 µM
EGTA, 7.5 mM	10µl	0.75 nM
Forward primer	var	150 nM
Tth Polymerase(5µ/µl)	0.8µl	4 units
Total ⁽¹⁾	80µl	
Total volume: ⁽²⁾	100µl	

Note

- 1) Gently vortex and add the 80 µl PCR master mix to the RT-PCR reaction (after incubation) at room temperature.
- 2) Continue cycling immediately! See next line.

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

Note:

- 1) Temperature depends on the melting temperature of the primer; approximately 5°C to 8°C below the T_m of primers.
- 2) We recommend adding 5 seconds per cycle extension.
- 3) It 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 50µl	
Forward primer	var.	400 µM
Reverse primer	var.	400 µM
Template RNA	var.	Up to 0.5µg
Total	50µl	

Mix II:

Component	Volume	Final conc.
Sterile water	Up to 50µl	
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	1-2min	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) The temperature depends on the melting temperature of the primer; approximately 5°C to 8°C below the temperature of primers.
- 2) We recommend adding 5 seconds per cycle extension.
- 3) It depends on the copy number of the RNA.

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