Tth DNA Polymerase

Item number: PC1170
Specification: 500U
Storage: -20℃ long-term storage, avoid repeated freezing and thawing.

Product Description:

Tth DNA Polymerase is a heat-stable DNA polymerase derived from Thermus thermophilus HB8. The enzyme can catalyze the polymerization of 5 'to 3' DNA template-dependent deoxy nucleotides in the presence of Mg^{2+}, and can be widely used in various PCR reactions. It is more resistant to blood-derived inhibitors than Taq. In the presence of Mn^{2+}, it showed stronger reverse transcriptional activity and could be used for single-tube one-step RT-PCR reaction. Tth DNA Polymerase developed by our company is a high-purity Tth DNA Polymerase product which is expressed by *E.oil* recombinant and obtained through a multi-step chromatography process.

The activity of the polymerase:

The amount of enzyme required to incorporate 10 nmol dNTPs into acid-insoluble DNA at 70 ° C for 30 minutes is 1 active unit (U).

Reagent composition:

1. Tth DNA Polymerase (5U/μL)
2. 2 x Tth RT Buffer (including Mn^{2+})

How to use:

Preparation of RT-PCR reaction system:

Component	Volume perReaction (μL)	Concentration in Master Mix
DNA or RNA	*	
Primer 1	1 ~ 5	0.2 ~ 1.0 microns
Primer 2	1 ~ 5	0.2 ~ 1.0 microns
dNTPs or 10mM each dNTP	1.5	From 200 to 300 microns
2×Tth RT Buffer a	25	1 x
Tth (5U/μL)	1 ~ 2	5.0 ~ 10.0U
ddH_2O	*	——
Total volume	50muL	

PCR reaction procedure - two step method:

Variability: 95℃ 3min;
Variability: 95℃ 10~20s;
Annealing/extension: 60℃ for 20 to 60s } 35~50 cycles

RT-PCR one-step reaction procedure -- specific primers:

reverse transcription: 60℃	30min;	
Variability: 95℃	3min;	
Variability: 95℃	10~20s;	35~50 cycles
annealing/extension: 60℃	20~60s	

Note:

1. With 5 '-3' polymerase, 5 '-3' exonuclease activity; No 3 '-5' exonuclease activity; The 3 'end of PCR product was A;
2. The commonly used reverse transcription temperature of Tth is 60℃, which can be optimized at 60℃ ~70 ℃ according to the characteristics of amplification reaction; The reverse transcription time can be optimized in 15~30min;
3. It is more suitable for RT reaction with specific primers, and the Tm value of primers should be 60℃ or higher; Primers with too low Tm value such as Oligo (dT) 18-20 or Random Primers are not recommended;
4. It can effectively improve the problem of low reverse transcription efficiency due to the complex secondary structure of RNA; And help to improve the specificity of prim-template hybridization;
5. Tth has better heat resistance and higher thermal stability compared with MMLV; Compared with Taq, TTH has higher tolerance to various PCR inhibitors;
6. Because Mn^{2+} was used for amplification, the fidelity of this system was reduced, and it was not suitable for cloning, sequencing and other experiments with high fidelity requirements.