Cat.: E17

Robustart Tth DNA Polymerase V01

Product Description

Robustart Tth DNA Polymerase is a hot-start Tth enzyme that has 5'-3' DNA template-dependent polymerase activity, making it suitable for various PCR reactions. It also has higher reverse transcriptase activity in the presence of Mn^{2+}, effectively eliminating the adverse effects of RNA secondary structures and non-specific primer annealing at low temperatures during cDNA synthesis, thereby increasing reverse transcription specificity and improving amplification efficiency and yield. It can be used for single-enzyme one-step RT-PCR reactions. Robustart Tth's activity is blocked at room temperature, which improves its specificity and significantly enhances the amplification effect of low-concentration templates.

Components

1. 5 U/μL Robustart Tth DNA Polymerase
2. 10×Tth RT Buffer Basic (Mn^{2+} free) (optional)
3. 25 mM Mn $(OAc)_2$ (optional)

*10×Tth RT Buffer Basic (Mn^{2+} free) does not contain dNTPs or Mg^{2+}. Please add dNTPs and $Mn(OAc)_2$ when preparing reaction systems.

Unit Definition

One unit of activity (U) refers to the amount of enzyme required to incorporate 10 nmol of deoxyribonucleotides into acid-insoluble material using activated salmon sperm DNA as a template/primer within 30 minutes at 74°C.

Storage

Store at -20±5℃. Vortex before use. Avoid freeze-thaw cycles.

Quality Control

1. SDS-PAGE electrophoresis purity no less than 98%.
2. Amplification sensitivity, between-batch difference, and stability.
3. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.

Prepare Reaction Mix

Components	Volume per Reaction	Final Concentration
10×Tth RT Buffer Basic (Mn^{2+} free) [1]	5 μL	1×
dNTPs (10 mM each)	1.5 μL	300 μM
25 mM $Mn(OAc)_2$	2-6 μL	1-3 mM
5 U/μL Robustart Tth DNA Polymerase	1-2 μL	5-10U/Reaction
25×Primer Mix [2]	2 μL	1×
Template	——	＜1 μg/Reaction
ddH_2O	To 50 μL	——

1. This Buffer does not contain dNTP or Mn^{2+}. dNTPs and $Mn(OAc)_2$ must be added before use.
2. If used for qPCR/qRT-PCR, fluorescent probes need to be added to the reaction system. The final concentration of primers is usually 0.2 μM for better results. when the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2-1 μM. The probe concentration is usually optimized in the range of 0.1-0.3 μM. Concentration gradient experiments can be performed to find the best combination of primers and probes.

Reaction Programe

Standard PCR Program			
Procedure	Temp	Time	Cycle
Hot Start	95°C	1-5 min	1
Denaturation	95°C	10-20 s	35-50
Annealing and Extension	56-64°C	20-60 s	

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One-step RT-PCR Program			
Procedure	Temp.	Time	Cycle
Denaturation	90°C	30 s	1
Reverse Transcription	60°C	25 min	1
Denaturation	95°C	1-5 min	1
Denaturation	95°C	10-20 s	35-50
Annealing and Extension	56-64°C	20-60 s	

Notes

1. Robustart Tth DNA Polymerase has 5′-3′ polymerase and exonuclease activities but no 3′-5′ exonuclease activity. PCR products end with A.
2. The commonly used reverse transcription temperature for Robustart Tth is 60℃, which can be optimized between 60℃-70℃ depending on the characteristics of the amplification reaction. Reverse transcription time can be optimized between 15-30 min.
3. It is more suitable for RT reaction with specific primers, and the Tm value of the primer should be 60℃ or higher. Primers with low Tm value such as Oligo (dT) $_{18\text{-}20}$ or Random Primers are not recommended.
4. It can effectively reduce the low reverse transcription efficiency caused by the complex secondary structure of RNA, and improve the specificity of primer-template hybridization.
5. Compared with M-MLV, it has higher thermal stability. And it has higher tolerance to various PCR inhibitors.
6. Due to the use of Mn^{2+}, the fidelity of amplification using Tth is reduced. So it is not suitable for cloning, sequencing and other experiments with high fidelity requirements.
7. Three-step PCR method is recommended for primers with low annealing temperature or for the amplification of long fragments over 200 bp.