Cat.:E03

AcuGenix™ Hot Start Taq DNA Polymerase V01

Product Description

AcuGenix™ Hot Start Taq DNA Polymerase is a hot-start Taq polymerase that employs a chemical modification method to completely block the enzyme's activity at room temperature. AcuGenix™ Hot Start Taq DNA Polymerase effectively suppresses non-specific amplification caused by non-specific annealing of primers or primer dimers at low temperatures, thereby improving the specificity and sensitivity of PCR reactions. It also adopts a "Time release" mechanism, whereby enzyme activity is gradually released during the temperature ramp-up of PCR cycles, further enhancing the specificity and sensitivity of low-copy template amplification. The main advantages of this enzyme for fluorescence PCR (real-time PCR) applications are high sensitivity, high fluorescence values, and high specificity.

Components

1. 5 U/μL AcuGenix™ Hot Start Taq DNA Polymerase
2. 10×PCR Buffer Ⅱ (Mg^{2+} free) (optional)
3. 25 mM $MgCl_2$ (optional)

*10×PCR Buffer Ⅱ (Mg^{2+} free) does not contain dNTPs or Mg^{2+}. Please add dNTPs and $MgCl_2$ 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 buffer

20 mM Tris-HCl (pH 8.0),100 mM KCl, 1 mM EDTA,1 mM DTT, Stabilizer, 50% Glycerol.

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×PCR Buffer Ⅱ (Mg^{2+} free) [1]	5 μL	1×
dNTPs (10 mM each)	1 μL	200 μM
25 mM $MgCl_2$	2-8 μL	1-4 mM
5 U/μL AcuGenix™ Hot Start Taq DNA Polymerase	0.25-0.5 μL	1.25-2.5U/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 Mg^{2+}, dNTPs and $MgCl_2$ 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

Two-step			
Procedure	Temperature	Time	Cycle
Hot Start	95℃	5-10 min	1
Denaturation	95℃	10-20 s	40-50
Annealing and Extension	56-64℃	20-60 s	

Three-step			
Procedure	Temperature	Time	Cycle
Hot Start	95℃	5-10 min	1
Denaturation	95℃	10-20 s	40-50
Annealing	56-64℃	10-30 s	
Extension	72℃	10-60 s	

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Notes

1. Due to differences in ramp rates among thermal cycles and the intrinsic properties of this enzyme, if a 5-minute hot-start step results in poor amplification, it is recommended to optimize the hot-start time within the range of 5 to 15 minutes.
2. With high specificity and sensitivity, it is suitable for multiplex PCR reactions.
3. The DNA polymerase has 5'-3' polymerase activity, 5'-3' exonuclease activity, lacks 3'-5' exonuclease activity or proofreading function.
4. Suitable for conventional PCR, RT-PCR, and detection methods such as probe-based assays, fluorescent dye-based assays, and gene chip assays.
5. The PCR product has an A at the 3' end and can be directly cloned into T vectors.
6. For primers with low annealing temperatures or amplicon fragments longer than 200 bp, it is recommended to use a three-step method for amplification.