

Product Description

AcuGenix™ Hot Start Taq DNA Polymerase(Glycerol-Free) is an enzyme optimized and formulated specifically according to the special requirements of freeze-dried reagents. The enzyme is a hot-start Taq polymerase that completely blocks Taq enzyme activity at room temperature using chemical modification. AcuGenix™ Hot Start Taq DNA Polymerase(Glycerol-Free) can effectively suppress non-specific amplification caused by primer annealing or primer dimerization at low temperatures, thereby improving the specificity and sensitivity of PCR reactions. It can also be used in a "time release" mode to gradually release enzyme activity during the PCR cycle, further increasing the specificity and sensitivity of amplification for low-copy templates. The main advantages of using this enzyme in fluorescent PCR reactions are high sensitivity, high fluorescence intensity, and high specificity.

Components

1. 5 U/μL AcuGenix™ Hot Start Taq DNA Polymerase(Glycerol-Free)
2. 10×HS Buffer (Mg²⁺ free) (optional)
3. 25 mM MgCl₂ (optional)

*10×HS Buffer (Mg²⁺ free) does not contain dNTPs or Mg²⁺. Please add dNTPs and MgCl₂ when preparing reaction systems.

Unit Definition

The amount of enzyme required to incorporate 10 nmol of deoxynucleotide into acid-insoluble material within 30 minutes at 74°C, using activated salmon sperm DNA as the template/primer, is defined as one unit of activity (U).

Storage Buffer

20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, Stabilizer.

Storage

Storage at -20±5°C. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Quality Control

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

Prepare the PCR Reaction Mix

Component	Volume per Reaction	Final Concentration
10×HS Buffer (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 (Glycerol-Free)	0.25-0.5 μL	1.25-2.5U
25×Primer Mix ²	2 μL	1×
Template	—	<1 μg / Reaction
ddH ₂ O	To 50 μL	—

1. This buffer does not contain dNTPs or Mg²⁺, so they must be added to the reaction system before use.
2. If used for qPCR/qRT-PCR, a fluorescent probe needs to be added to the reaction system. Typically, a final primer

concentration of 0.2 μ M performs good results; if the reaction performance is poor, adjust the primer concentration within the range of 0.2-1 μ M. Typically, probe concentrations are optimized in the range of 0.1-0.3 μ M. Combinations of primers and probes can be tested using gradient experiments to find their optimal combination.

Thermocycling conditions

Two-step Method				Three-step Method			
Procedure	Temp.	Time	Cycle	Procedure	Temp.	Time	Cycle
Denaturation	95°C	5~10 min	1	Denaturation	95°C	5~10 min	1
Denaturation	95°C	10~20 s	35~50	Denaturation	95°C	10~20 s	40
Annealing and Elongation	56~64°C	20~60 s		Annealing and Elongation	56~64°C	10~30 s	
				Extension	72°C	10~60 s	

Notes

1. Due to differences in temperature control performance among different instruments and its own characteristics, the effect of amplification by five-minute hot start may not be ideal. It is recommended that hot-start time should be optimized within a range of 5~15 min.
2. High specificity and sensitivity make it suitable for multiplex PCR reactions.
3. Performs both 5'-3' polymerase activity and exonuclease activity; no proofreading function or 3'-5' exonuclease activity.
4. Suitable for ordinary PCR, RT-PCR as well as detection methods such as probe method, fluorescence dye method, and gene chip method.
5. PCR products have an A at their 3' end which allows direct T-vector cloning.
6. For primers with low annealing temperatures or amplicons over than 200 bp, three-step cycling is recommended.