Hotstart Taq DNA Polymerase

Cat.No.: HYA113

Product Description

This product not only effectively inhibits nonspecific reactions caused by primer nonspecific annealing or primer dimers during PCR system preparation and amplification, thereby delivering excellent specificity. It enables more efficient amplification of low-concentration templates and is suitable for multiplex PCR amplification reactions. Additionally, it exhibits outstanding compatibility with various reaction systems, consistently providing stable amplification performance across different types of PCR reactions.

Components

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

*10×PCR Buffer (Mg^{2+} free) does not contain dNTP and Mg^{2+}, please add dNTPs and $MgCl_2$ when preparing the reaction system.

Activity Definition

One activity unit (U) is defined as the amount of enzyme required to incorporate 10 nmol of deoxynucleotides into acid-insoluble material, using activated salmon sperm DNA as the template/primer, under the conditions of 74℃ for 30 minutes.

Storage

Store at -20℃ for long-term storage. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Quality Control

1. Electrophoretic purity by SDS-PAGE ≥ 98%.
2. Amplification sensitivity, inter-batch variation, and stability.
3. Free of extraneous nuclease activity, as well as extraneous endonuclease and exonuclease contamination.

Instructions

1. PCR reaction system

Components	Volume	Concentration in Master Mix
10 × PCR Buffer (Mg^{2+}free)*	5 uL	1×
dNTPs（10 mM each dNTP）	1 μL	200 μM
25 mM $MgCl_2$	2-8 μL	1-4 mM
5U/μL Hotstart Taq DNA Polymerase	0.25-0.5 μL	1.25-2.5 U
25×Primer Mix**	2 μL	1×
Template	— —	< 1 μg/reaction
ddH_2O	Up to 50 μL	— —

*10 × PCR Buffer does not contain dNTPs or Mg^{2+}, and must be supplemented with dNTPs and $MgCl_2$ before use.

**If used for qPCR/qRT-PCR, a fluorescent probe must be added to the reaction system. Typically, a final primer concentration of 0.2 μM yields optimal results; if the reaction performance is unsatisfactory, the primer concentration can be adjusted within the range of 0.2–1 μM. Generally, the probe concentration is optimized within 0.1–0.3 μM. Concentration gradient experiments can be performed to identify the optimal combination of primers and probes.

2. Reaction Program

Two-step method			
Step	**Temperature**	**Time**	**Cycles**
Hot start	95 ℃	1-5 min	1
Denaturation	95 ℃	10-20 s	35-50
Annealing-Extension	56~64℃	20-60 s	

Three-step method			
Step	**Temperature**	**Time**	**Cycles**
Hot start	95 ℃	1-5 min	1
Denaturation	95 ℃	10-20 s	35-50
Annealing	56~64℃	10-30 s	
Extension	72℃	10-60 s	

Technical Specifications

1. Enables rapid hot start at 95℃ or 94℃ for 1–5 minutes.
2. Exhibits excellent compatibility with reaction systems, along with enhanced specificity and sensitivity.
3. Suitable for high-sensitivity PCR detection reagents and compatible with multiplex PCR amplification reactions.
4. Possesses 5’-3’ polymerase activity and 5’-3’ exonuclease activity; lacks 3’-5’ exonuclease activity and proofreading function.
5. Applicable for qualitative and quantitative detection in conventional PCR and RT-PCR.
6. PCR products feature an A-overhang at the 3’ end, allowing direct use for T-vector cloning.
7. For primers with low annealing temperatures or amplification of long fragments exceeding 200 bp, it is recommended to use the three-step PCR protocol.
8. For Research Use Only!