Tribioscience

Hotstar DNA Polymerase

Catalog	Unit
TBP0152-1000	1000 U

Description

Hotstar DNA Polymerase is a specialized enzyme blend designed for Hot Start PCR applications. It consists of a monoclonal anti-Taq antibody and Taq DNA Polymerase, working together to suppress DNA polymerase activity at lower temperatures. By binding to the Taq enzyme, the antibody effectively prevents non-specific primer annealing and amplification caused by primer dimers. With its rapid extension capability and high amplification efficiency, Hotstar DNA Polymerase is ideal for DNA fragment amplification, sequence analysis, and various other molecular biology applications.

Product Details

Purity: 99% by SDS-PAGE
Storage: -20°C

Components

Component	1000 U
Hotstar DNA Polymerase, 5U/μL	2×100μl
10× PCR Buffer	2×1.8ml

Note: The 10× PCR Buffer of this product contains 15 mM magnesium ions.

Protocol

The following example is a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as template. In actual operation, corresponding improvement and optimization should be carried out according to the different template, primer structure and target fragment size.

1. PCR Reaction System

Reagent	50μL Reaction System	Final Concentration
10× PCR Buffer	5μL	1×
dNTP Mix, 10mM each	1μl	200μM each
Forward Primer, 10μM	2μl	0.4μM
Reverse Primer, 10μM	2μl	0.4μM
Template DNA	<0.5μg	< 0.5μg/50μL
Hotstar DNA Polymerase, 5U/μL	0.25-0.5μL	1.25-2.5 U/50μL
ddH2O	up to 50μl	

Note: The reaction solution can be prepared at room temperature, and the reagents must be kept on ice.

2. PCR Reaction Condition

Step	Temperature	Time	Cycles
Pre denaturation	94°C	2min	
Denaturation	94°C	30s	25-35cycles
Annealing	55-65°C	30s	
Extend	72°C	30s	
Final extension	72°C	2min	

Note:

1) In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer, and when the ideal amplification efficiency cannot be obtained, the annealing temperature should be appropriately reduced.
2) The extension time should be set according to the size of the amplified fragment.
3) The number of cycles can be set according to the downstream application of the amplified product. If the number of cycles is too small, the amount of amplification will be insufficient; if the number of cycles is too many, the probability of mismatching will increase and the non-specific background will be severe. Therefore, the number of cycles should be minimized on the premise of ensuring the product yield.

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