Tinzyme Co., Limited

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Hotstar DNA Polymerase

Product Number: PC11

Shipping and Storage

-20°C.

Components

Component	PC11 500U	PC11 2500U
Hotstar DNA Polymerase, 5U/μL	100μl	5×100μl
10× PCR Buffer	1.8ml	5×1.8ml

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

Description

Hotstar DNA Polymerase is a mixture of anti-Taq monoclonal antibody and PC01 Taq DNA Polymerase, suitable for Hot Start PCR. When using Taq enzyme antibody for PCR amplification, the binding of Taq enzyme antibody to Taq enzyme inhibits DNA polymerase activity before high temperature denaturation, which can effectively inhibit the non-specific annealing of primers and non-specific amplification caused by primer dimers under low temperature conditions. The Taq enzyme antibody is neutral in the initial DNA denaturation step of the PCR reaction, and the DNA polymerase activity is restored to achieve the hot start effect.The use of this product does not require special inactivation of Taq enzyme antibody, and can be used under conventional PCR reaction conditions.

Hotstar DNA Polymerase has 5′ →3′ DNA polymerase activity and 5′→3′ exonuclease activity, but no 3′→5′ exonuclease activity. The enzyme extension speed is 2 kb/min, and it can amplify up to 5 kb in length fragment. The amplified PCR product has an "A" base attached to the 3' end, so it can be used directly for T/A cloning. This product has the characteristics of fast extension speed and high amplification efficiency, and is mainly suitable for PCR amplification of DNA fragments, DNA sequence determination and other experiments.

Unit definition

Using activated salmon sperm DNA as template/primer, the amount of enzyme required to incorporate 10 nmol of deoxynucleotides into acid-insoluble substances at 74°C for 30 minutes was defined as 1 activity unit (U).

Quality Control

After several column purifications, the purity of SDS-PAGE test is more than 99%; no exogenous nuclease activity is detected; no host residual DNA is detected by PCR method; it can effectively amplify single-copy genes in the human genome.

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 Conc.
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
ddH_2O	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	
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.