



Hotstar DNA Polymerase

Product Number: PC11

Shipping and Storage

Ice pack transportation; Stored at -20°C, with a shelf life of 2 years, to avoid repeated freezing and thawing.

Components

Component	200U	1KU	5KU
Hotstar DNA Polymerase, 5U/μL	40μL	200μL	1mL
10× Reaction Buffer (Mg ²⁺ plus)	200μL	1mL	5mL

Description

HS Taq DNA polymerase is a chemically modified Taq DNA polymerase, whose activity is completely blocked at room temperature and only released after heating at 95 °C, which can prevent non-specific amplification and primer dimerization during sample preparation and reaction heating stages. Compared with antibody based hot start Taq enzymes, HS Taq DNA polymerase activity is more thoroughly blocked and has higher rigor; Compared with existing chemically modified hot start Taq enzymes, the activation time of HS Taq DNA polymerase is only 5 minutes, which is compatible with existing PCR programs. HS Taq DNA polymerase, combined with an optimized buffer system, can minimize non-specific amplification and primer dimers to the greatest extent possible, bringing extremely high sensitivity and specificity, making it very suitable for amplifying low copy genes from complex templates.

Application

Mainly used for DNA amplification reactions in animals, plants, and microorganisms. Hot start enzymes have 5'-3' exonuclease activity and can be used for fluorescence quantitative PCR reactions. The use of hot start amplification is a common method to improve PCR specificity, and hot start enzymes are a good choice. In addition, due to its high specificity and sensitivity, it is widely used in various fields such as constructing cDNA libraries, generating large amounts of DNA sequencing, mutant analysis and construction, gene isolation, genetic disease diagnosis, forensic identification, etc.

Unit definition

The activity of consuming 10 nmol of whole nucleotide as an acidic insoluble substance within 30 minutes at 74°C using activated salmon sperm DNA as a template/primer is defined as one activity unit (U).

Protocol

1. Reaction System

Reagent	Volume
10× Reaction Buffer (Mg ²⁺ plus)	5μL
dNTP Mix, 10mM each	1μL
Primer 1, 10μM	2μL
Primer 2, 10μM	XμL
Template DNA	2μL
Hotstar DNA Polymerase, 5U/μL	0.5-0.1μL
ddH ₂ O	up to 50μL

The optimal reaction concentration varies for different templates. The following table shows the recommended template usage for a 50μL reaction system:

Template	Usage
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Genomic DNA of animals and plants	0.1 - 1 μ g
Escherichia coli genomic DNA	10 - 100ng
cDNA	1-5 μ L (not exceeding 1/10 of the total volume of PCR reaction)
Plasmid DNA	0.1 - 10ng
λ DNA	0.5 - 10ng

The enzyme concentration can be adjusted between 0.25-1 μ L. Increasing the enzyme amount can usually increase amplification yield, but it may lead to a decrease in specificity.

2. Reaction procedure

Temperature	Time	Cycle
95 $^{\circ}$ C	5min(Pre denaturation) ^a	
95 $^{\circ}$ C	10-30s	} 25-35cycles
55-60 $^{\circ}$ C ^b	30s	
72 $^{\circ}$ C	20-30s/kb	
72 $^{\circ}$ C	5min(Completely extended)	

a. This pre denaturation condition is suitable for the vast majority of amplification reactions and can be modified according to the complexity of the template structure. If the template structure is complex, the pre denaturation time can be extended to 5-10 minutes to improve the pre denaturation effect;

b. The annealing temperature needs to be adjusted according to the T_m value of the primer, generally set to 3-5 $^{\circ}$ C lower than the T_m value of the primer; For complex templates, it is necessary to adjust the annealing temperature and extend the extension time to achieve efficient amplification.

Note

1. This product is only for scientific research purposes.
2. Suggest separately packaging and storing to avoid repeated freezing and thawing.
3. For your safety and health, please wear lab coats and disposable gloves when operating.