HotStart Taq DNA Polymerase

For research use only

Catalogue Numbers: HS005, HS050, HS250, HSN005, HSN050, HSN250

Quantity: 50 U, 500 U, 2500 U

Unit Definition: 1 U incorporates 10 nmole of dNTP into acid insoluble products in 30 minutes at 74ºC

Storage Buffer: 20 mM Tris-HCl pH8.0, 0.1 mM EDTA, 1 mM DTT, 1.0% Triton X-100, 50% Glycerol

10X PCR Buffer II: 200 mM Tri-HCl, pH 8.1, 40 mM $MgCl_2$, 100 mM KCl, 1.0% Triton X-100

$MgCl_2$: premixed with 10X PCR Buffer

Storage: -20ºC for extended periods

3' to 5' Exonuclease Poofreading Abiliy: NO

5' to 3' Exonuclease Activity: YES

Introduction

HotStart Taq DNA Polymerase is a thermostable, inhibitor modified form of recombinant Taq DNA Polymerase with ultra low DNA content. It is inactive at temperatures below 45ºC, but is activated at 95ºC (1 minute). HotStart Taq DNA Polymerase is ideal for "Hot Start" qPCR, because the enzyme remains inactive during qPCR set-up. Since it is inactive at low temperatures, it does not elongate non-specific primer-template hybrids that may form at low temperatures. The enzyme will amplify DNA targets with high specificity, sensitivity, and yield.

Quality Control

HotStart Taq DNA Polymerase is tested on a lot-to-lot basis. Quality control assays include activity test, PCR, endonuclease activity, exonuclease activity.

Caution

During the procedure, always wear a lab coat, disposable gloves, and protective goggles

Component	HS005/HSN005	HS050/HSN050	HS250/HSN250
HS Taq DNA Polymerase	10 µl (50U)	100 µl (500U)	100 µl x 5 (2500U)
10X PCR Buffer II	200 µl	2 ml	2 ml x 5
dNTPs (10 mM each)	NA/20 µl	NA/200 µl	NA/1 ml

Advantages

Simplified "Hot Start" Technique: The modified Taq DNA Polymerase is inactive at room temperature but is readily activated during the first high temperature step of PCR.

Fast Activation (95ºC for 1 minute): Other Hot Start Taq requires 4 to 10 minutes of heating to activate the enzyme. Extended heating time reduces the duration of Taq activity and can delay the cycle threshold (Ct).

Increased PCR Specificity: The enzyme will not amplify non-specific, low-temperature primer-template hybrids.

Increased PCR Sensitivity & Yield: The enzyme leads to a more specific product.

Improved Performance of Multiplex PCR: Eliminates non-specific priming, resulting in higher specificity/sensitivity, and lower non-specific background signals.

Ideal for High Throughput or Automated PCR: Reactions can be set up at room temperature.

Reduces Risk of Contaminant Carry-over: Allows "Hot Start" without additional pipetting/handling steps or additional reaction components such as wax.

HotStart Taq PCR Reaction Set Up

All reaction components should be prepared on ice or in a biocooler then quickly transferred to a thermal cycler pre-heated to 95ºC. For added convenience and to prevent pipetting errors, combine the reaction components in a PCR master mix then aliquot several reactions in parallel PCR tubes. Storage Buffer contains 50% Glycerol so HotStart Taq DNA Polymerase does not require thawing when removed from -20ºC storage.

1. Allow components to thaw then vortex gently and spin down.
2. Remove HotStart Taq DNA Polymerase from -20ºC storage and spin down.
3. Add the following components to a PCR tube or tubes.

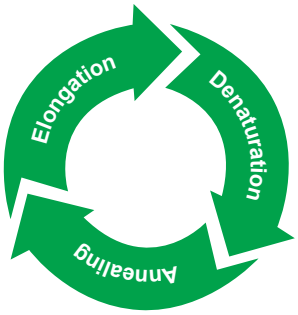
Reaction Components	Volume (µl)	Amount
Template DNA	Variable	<500 ng
Upstream Primer (10 µM)	0.2-1.0 µl	0.1-0.5 µM
Downstream Primer (10 µM)	0.2-1.0 µl	0.1-0.5 µM
dNTP Mix (10 mM each)	0.4 µl	200 µM
10X PCR Buffer II	2 µl	4 mM $MgCl_2$
HS Taq DNA polymerase	0.2 µl	1 Unit
ddH_2O	Add to 20 µl	

General Thermal Cycling Program

Please read the entire instruction manual prior to starting the thermal cycling program.

Initial Denaturation	• 95ºC. • 2-5 minutes. *Denaturation condition varies depending on thermal cycler and tube.
Step 2 Denaturation	• 95ºC. • 20-40 seconds.
Step 3 Annealing	• 55ºC-60ºC. • 30-60 seconds. • Optimal annealing temperature is dependent on the Tm value of the primers and the reaction condition.
Step 4 Elongation	• 72ºC • 20 seconds (for amplicon <500 bp). • For large amplicon PCR, add 30 seconds for each kbp.
Step 5 Final Elongation	• 72ºC. • 7 minutes.
Step 6 Cooling	• 4ºC.

30-40 Cycles



Patent Disclaimer

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