5 PRIME HotMaster® Taq DNA Polymerase

Cat. Nos.:	2200300	Size:	100 U (5u/µL)	**Store at -20ºC**
	2200320		1000 U (5u/µL)	
	2200330		5000 U (5u/µL)	

Description

The HotMaster Taq DNA Polymerase is a superior alternative for performing hot-start PCR. The HotMaster Taq DNA Polymerase formulation consists of a combination of 5 PRIME's Taq Polymerase and the proprietary HotMaster inhibitor (patent pending). This multipotent competitive polymerase inhibitor was discovered by screening a combinatorial library of derivatized natural affinity ligands of DNA polymerases. HotMaster blocks the substrate-binding site of DNA polymerases in a temperature-dependent manner. Inactive polymerase-inhibitor complexes are formed at temperatures < 40°C, where the affinity of HotMaster for Taq polymerase is higher than the binding affinity of the template DNA. Between 40°C and 55°C the HotMaster competes with the template DNA for binding to the Taq polymerase, shifting the binding equilibrium towards complex formation with only target-specific primed template DNA. At temperatures above 55°C, the HotMaster inhibitor is displaced from complexes with the Taq polymerase by target-specific primed template DNA. A unique performance feature of the HotMaster inhibitor is that it can go through multiple temperature cycles of binding-equilibrium competition–dissociation during PCR without irreversible heat inactivation. Where other Taq polymerase formulations for hot-start PCR only block the activity of Taq polymerase prior to the first high temperature step, the 5 PRIME HotMaster provides sustained temperature control throughout PCR.

Superior features for hot-start PCR applications

- → Does not require heat activation
- → Gives continuous annealing temperature control throughout the PCR
- → Extends target size range for PCR amplification (up to 5 kb)
- → Contains pre-optimized universal magnesium concentration in the buffer
- → Ensures no protein contamination of the PCR by denatured antibodies

Enzyme concentration

The enzyme concentration is 5 U/µl*

* One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 74°C under the assay conditions, using reaction conditions: 25 mM TAPS (N-tris-(hydroxy-methyl)-methyl-3-amino-propanesulfonic acid, sodium salt) at pH 9.3 and 25°C; 50 mM KCl; 2 mM $MgCl_2$; 1 mM ß-mercaptoethanol; 200 µmol each dATP, dGTP, dTTP; 100 µM dCTP (a mix of unlabeled and [α^{32}P]-labeled); and 12.5 µg activated salmon sperm DNA, in a final volume of 50 µl.

Enzyme storage buffer composition

25 mM Tris-HCl (pH 8).0, 35 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween 20, 0.5% IGEPAL, CA-630 and stabilizers.

Components

Component	100 U	1000 U	5000 U
Ordering number	2200300	2200320	2200330
HotMaster Taq DNA Polymerase (5 U/µl)	20 µl	4 x 50 µl	20 x 50 µl
10x HotMaster Taq Buffer with 25 mM Mg^{2+}	1.8 ml	3 x 1.8 ml	15 x 1.8 ml

Storage and Stability

The HotMaster Taq DNA Polymerase kits are shipped on dry ice and should be stored immediately upon receipt at –20°C in a constant-temperature freezer.

Quality Control

5 PRIME products are manufactured using quality chemicals and materials that meet our high standards. All product components are subjected to rigorous quality assurance testing process:

- Component testing: each component is tested to ensure the composition and quality meet stated specifications.
- Performance testing: each product is tested to ensure it meets the stated performance specification.

Additional quality information is available from Quantabio. Certificate of analysis for 5 PRIME products and 5 PRIME product components can be obtained on request.

Protocol

For a 50 µl reaction, mix the components listed in Table 1 at ambient temperature in a thin-walled PCR tube.

Initial template denaturation should be performed at 94°C for no more than 2 minutes. HotMaster Taq DNA Polymerase does not require heat activation.

The magnesium concentration does not need to be adjusted. The concentration in the HotMaster Taq buffer has been optimized for all targets. The optimal concentrations of other variable reaction components such as template DNA, enzyme, and primer must be determined empirically.

The recommended synthesis temperature for the primer elongation step in a PCR cycle is 65°C in an allowed range of 60°C to 70°C.

The optimal primer elongation temperature for quantitative real-time PCR with TaqMan® probes is 60°C.

Please see Table 2 for suggested cycling parameters.

Table 1. Components for 50 µl reaction.

Component	Volume	Final concentration
Molecular biology-grade water	Variable	
10x HotMaster Taq Buffer with Mg2+	5 µl	1x (2.5 mM Mg2+)
10 mM dNTP Mix	1 µl	0.2–0.25 mM
Primer A (forward)	Variable	0.1–0.5 µM
Primer B (reverse)	Variable	0.1–0.5 µM
Template DNA	Variable	0.1–200 ng
HotMaster Taq DNA Polymerase	0.25–0.5 µl	1.25–2.5 U
Total volume	**50 µl**	

Table 2. Suggested cycling parameters

PCR cycle	Temperature	PCR product size		
		100-500 bp	500-1000 bp	1-5 kb
Initial denaturation	94°C	2 min	2 min	2 min
Cycled template denaturation	94°C	20 sec	20 sec	20 sec
Cycled primer annealing	50–70°C	10 sec	10 sec	20 sec
Cycled primer extension	60–70°C	20–30 sec	40–50 sec	1 min/kb

Limited Label Licenses

For Research Purposes Only.

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