Manual HotMaster™ Taq DNA Polymerase

For highly specific hot-start PCR
Introduction

The HotMaster™ Taq DNA Polymerase Kit is a superior alternative for performing PCR experiments generally known as “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, thereby 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 block the activity of Taq polymerase only prior to the first high temperature step, the 5 PRIME HotMaster™ provides sustained temperature control throughout PCR.

Superior product features for “hot start” PCR applications

- HotMaster™ Taq Polymerase does not require heat activation
- Continuous annealing temperature control throughout the PCR
- Extended target size range for PCR amplification (up to 5 kb)
- Pre-optimized universal magnesium concentration in the buffer
- No protein contamination of the PCR by denatured antibodies

Materials

Materials supplied with the kits:

<table>
<thead>
<tr>
<th>Component</th>
<th>HotMaster™ Taq DNA Polymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 U</td>
</tr>
<tr>
<td>HotMaster™ Taq DNA Polymerase (5 U/µl)</td>
<td>20 µl</td>
</tr>
<tr>
<td>10x HotMaster™ Taq Buffer with 25 mM Mg²⁺</td>
<td>1.8 ml</td>
</tr>
</tbody>
</table>
Enzyme concentration

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) pH 9.3 (at 25°C); 50 mM KCl; 2 mM MgCl2; 1 mM β-mercaptoethanol; 200 µmole each dATP, dGTP, dTTP; 100 µM dCTP (a mix of unlabeled and [α32P]-labeled); 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.

Storage and stability

Store at –20°C in a constant temperature freezer. If stored as recommended the kit is stable at least until the expiration date on the label.

Quality assurance

Each lot of HotMaster™ Taq DNA Polymerase is performance tested with human genomic DNA to amplify a specific 3 kb product from the human β-globin gene and a 131 bp product from the human TNF gene.

Endo- and Exonuclease activities were not detectable after overnight incubation, respectively, of 1 µg supercoiled plasmid DNA and 1 µg of Hind III digested Lambda DNA at 37°C in the presence of 15 to 20 units of HotMaster™ Taq DNA Polymerase.
Protocol

For a 50 µl reaction, mix the following components at ambient temperature in a thin-walled PCR tube:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Biology Grade Water</td>
<td>variable</td>
<td></td>
</tr>
<tr>
<td>10x HotMaster™ Taq Buffer with Mg²⁺</td>
<td>5 µl</td>
<td>1x (2.5 mM Mg²⁺)</td>
</tr>
<tr>
<td>10 mM dNTP Mix</td>
<td>1 µl</td>
<td>0.2 - 0.25 mM</td>
</tr>
<tr>
<td>Primer A (forward)</td>
<td>variable</td>
<td>0.1 – 0.5 µM</td>
</tr>
<tr>
<td>Primer B (reverse)</td>
<td>variable</td>
<td>0.1 – 0.5 µM</td>
</tr>
<tr>
<td>Template DNA</td>
<td>variable</td>
<td>0.1 – 200 ng</td>
</tr>
<tr>
<td>HotMaster™ Taq DNA Polymerase</td>
<td>0.25 – 0.5 µl</td>
<td>1.25 – 2.5 U</td>
</tr>
<tr>
<td>Total volume</td>
<td>50 µl</td>
<td></td>
</tr>
</tbody>
</table>

Initial template denaturation should be performed at 94°C for no more than 2 minutes, HotMaster™ Taq 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 sequence-specific probes is 60°C.
Suggested cycling parameters:

<table>
<thead>
<tr>
<th>PCR Cycle</th>
<th>Temperature</th>
<th>100-500 bp</th>
<th>500-1000 bp</th>
<th>1 - 5 kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94°C</td>
<td>2 min</td>
<td>2 min</td>
<td>2 min</td>
</tr>
<tr>
<td>Cycled template denaturation</td>
<td>94°C</td>
<td>20 sec.</td>
<td>20 sec.</td>
<td>20 sec.</td>
</tr>
<tr>
<td>Cycled primer annealing</td>
<td>50-70°C</td>
<td>10 sec.</td>
<td>10 sec.</td>
<td>20 sec.</td>
</tr>
<tr>
<td>Cycled primer extension</td>
<td>60-70°C</td>
<td>20-30 sec.</td>
<td>40-50 sec.</td>
<td>1 min/kb</td>
</tr>
</tbody>
</table>
## Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>Package Size</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HotMaster™ Taq DNA Polymerase</td>
<td>100 U</td>
<td>2200300</td>
</tr>
<tr>
<td>HotMaster™ Taq DNA Polymerase</td>
<td>250 U</td>
<td>2200310</td>
</tr>
<tr>
<td>HotMaster™ Taq DNA Polymerase</td>
<td>1000 U</td>
<td>2200320</td>
</tr>
<tr>
<td>HotMaster™ Taq DNA Polymerase</td>
<td>5000 U</td>
<td>2200330</td>
</tr>
<tr>
<td>MasterTaq™ Kit</td>
<td>100 U</td>
<td>2200200</td>
</tr>
<tr>
<td>MasterTaq™ Kit</td>
<td>250 U</td>
<td>2200210</td>
</tr>
<tr>
<td>MasterTaq™ Kit</td>
<td>500 U</td>
<td>2200220</td>
</tr>
<tr>
<td>MasterTaq™ Kit</td>
<td>1000 U</td>
<td>2200230</td>
</tr>
<tr>
<td>5 PRIME MasterMix</td>
<td>100 Rxns</td>
<td>2200100</td>
</tr>
<tr>
<td>5 PRIME MasterMix</td>
<td>1000 Rxns</td>
<td>2200110</td>
</tr>
<tr>
<td>Deoxynucleotide Mix - 10 mM</td>
<td>200 µl</td>
<td>2201200</td>
</tr>
<tr>
<td>Deoxynucleotide Mix - 10 mM</td>
<td>1000 µl</td>
<td>2201210</td>
</tr>
<tr>
<td>Deoxynucleotides Set</td>
<td>4 x 100 µl</td>
<td>2201220</td>
</tr>
<tr>
<td>Deoxynucleotides Set</td>
<td>4 x 250 µl</td>
<td>2201230</td>
</tr>
<tr>
<td>10x Taq Buffer, with 15 mM Magnesium</td>
<td>1.8 ml</td>
<td>2201240</td>
</tr>
<tr>
<td>5x TaqMaster PCR Enhancer</td>
<td>1.7 ml</td>
<td>2201250</td>
</tr>
<tr>
<td>Water, Mol Bio grade</td>
<td>1 l</td>
<td>2500000</td>
</tr>
<tr>
<td>Water, Mol Bio grade</td>
<td>10 x 50 ml</td>
<td>2500010</td>
</tr>
<tr>
<td>Water, Mol Bio grade</td>
<td>5 l</td>
<td>2500020</td>
</tr>
</tbody>
</table>
5 PRIME Distributors

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