5 PRIME HotMasterMix

Cat. No.: 2200400 Size: 100 Rxn (2 x 1 ml) **Store at -25°C to -15°C**
2200410 1000 Rxn (20 x 1 ml)

Description

5 PRIME HotMasterMix is a 2.5x concentrated, ready-to-use reagent mix for performing methods classified as hot-start PCR with minimal pipettings steps. The kit contains all reagents necessary to perform the polymerase chain reaction except primer and template DNA. The key component of the reagent mix is the HotMaster Taq DNA Polymerase which 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. Uniquely, the 5 PRIME HotMasterMix provides sustained temperature control throughout PCR. Temperature control is achieved because the HotMaster inhibitor can go through multiple temperature cycles of binding-equilibrium competition–dissociation and because the Taq DNA polymerase is not irreversibly activated during the first high temperature step as with other hot-start polymerases. The 5 PRIME HotMasterMix buffer is specially formulated to adjust the Mg^{2+} concentration in the reaction automatically. This feature eliminates the need for optimization of this critical component. The HotMasterMix buffer contains a component that weakly chelates Mg^{2+} ions. When Mg^{2+} is present in the reaction in excess, the Mg^{2+} remains bound to the chelating agent. As Mg^{2+} is needed in the reaction, the chelating agent releases it.

Components

Component	100 Rxn	1000 Rxn
Ordering number	2200400	2200410
2.5x HotMasterMix Solution	2 x 1 ml	20 x 1 ml

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

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 www.5PRIME.com. Certificate of analysis sheets for 5 PRIME products and 5 PRIME product components can be obtained on request.

Protocol

PCR is a sensitive technique capable of amplifying trace amounts of DNA, but also sensitive to cross-contamination from the environment. Ideally, the amplification reactions should be set up in a DNA-free environment using aerosol-resistant barrier tips. Analysis of the PCR products should take place in an area separated from the place where the reactions are assembled. Avoid contamination of the 5 PRIME HotMasterMix with primers and template DNA used in individual reactions.
Superior product features for hot-start PCR applications include:

No heat activation of the polymerase hot-start required
Easy handling with minimal pipetting steps
Extended target size range up to 5 kb
Self-adjusting universal magnesium concentration
No protein contamination by denatured antibodies

Before starting

Mix the 5 PRIME HotMasterMix thoroughly to avoid localized differences in salt concentrations.

Procedure

1.Prepare the template/primer mix in a 0.2 ml tube by adjusting the total volume to the values given in Table 1 with molecular biology-grade water.
2. Dispense the appropriate volume of the 5 PRIME HotMasterMix into a PCR tube (e.g., 20 µl for a 50 µl PCR reaction).
3. Add the template/primer mix to the PCR tube containing the HotMasterMix. Close the tube and mix well. If necessary, centrifuge briefly to collect liquid at bottom of tube.
4. Start the PCR program on a thermal cycler. The thermal cycler should be preheated (>90°C) before placing the PCR tube(s) on the cycler block.

Initial template denaturation should be performed at 94°C for no longer than 2 minutes. The HotMaster Taq DNA Polymerase does not require heat activation.
The magnesium concentration in the HotMasterMix is self-adjusting (up to a maximum of 2.5 mM) for all targets and does not need to be adjusted. The optimal concentrations of other variable reaction components such as template DNA 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 60–70°C.
See Table 2 for suggested cycling parameters.´

Table 1. PCR components for various reaction volumes

Component	Reaction volume 50 µl	Reaction volume 25 µl	Reaction volume 20 µl	Final concentration
Template/primer mix	30 µl	15 µl	12 µl	
Forward primer	Variable	Variable	Variable	100–200 nM
Reverse primer	Variable	Variable	Variable	100–200 nM
Template DNA	Variable	Variable	Variable	50 pg – 200 ng gDNA or 10 pg – 20 ng episomal DNA
Molecular biology grade water	Up to 30 µl	Up to 15 µl	Up to 12 µl	
5 PRIME HotMasterMix (2.5x)	20 µl	10 µl	8 µl	1x including 2.5 mM Mg2+

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

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