

Hot-Start PCR

HotMaster™ *Taq* DNA Polymerase



Innovative Hot-Start/Cold-Stop® technology for fast and specific Hot-Start PCR

- ⇒ Optimal results – highly specific amplification
- ⇒ Save time and effort – optimal PCR conditions provided
- ⇒ Excellent for fast PCR protocols – no enzyme activation
- ⇒ Also available as convenient HotMasterMix

Optimal results with highly specific amplification

Hot-start is a well established method for improving PCR specificity. HotMaster *Taq* DNA Polymerase is designed to reduce or eliminate any non-specific products that result from mispriming during PCR. Conventional hot-start technologies, such as antibody-mediated inhibition or chemical blocking of DNA polymerases, have limitations, such as long initial activation steps that can reduce the performance of the DNA polymerase and compromise specificity.

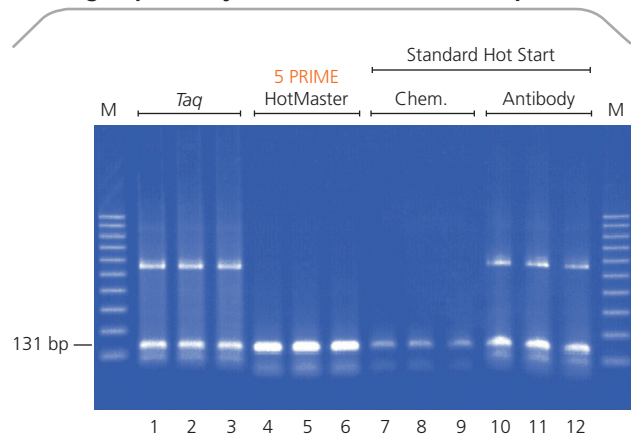
Hot-Start/Cold-Stop

Both HotMaster *Taq* DNA Polymerase and 5 PRIME HotMasterMix feature a superior hot-start PCR technology – Hot-Start/Cold-Stop®. A temperature-dependent inhibitory ligand completely inhibits *Taq* polymerase activity at low temperatures. At high temperatures, the inhibitor is released and full *Taq* activity is immediately restored. Since the process is reversible, the “cold-stop” aspect of the inhibitor has the potential to block enzyme activity in every low-temperature cycle of the PCR, ensuring optimal results. Unlike standard *Taq* polymerases, HotMaster *Taq* reactions can be set up at room temperature, eliminating time-consuming freeze-thaw cycles.

Optimized PCR conditions save time and effort

The HotMaster *Taq* DNA Polymerase is provided with a 10x self-adjusting Mg²⁺ buffer. The formulation adjusts the Mg²⁺ concentration automatically, eliminating the need to optimize this critical component. The self-adjusting buffer acts by weakly chelating Mg²⁺ ions: excess Mg²⁺ is bound by the chelating agent and, as free Mg²⁺ decreases, is released and available to the *Taq* polymerase. The self-adjusted Mg²⁺ concentration is pre-optimized for any template-primer system, saving time and precious samples.

High Specificity with Hot-Start/Cold Stop



Fast PCR Amplification of a 131 bp fragment of the human TNF gene with standard *Taq*, HotMaster *Taq* and conventional Hot Start enzymes. PCR protocol: 1 sec 95°C denaturation, 1 sec 55°C annealing, 5 sec 72°C extension. Initial denaturation was 2 min at 95°C prior to PCR or 10 min for the chemically modified enzyme respectively.

Excellent for fast PCR protocols – no enzyme activation

The initial heat-activation step required by standard hot-start PCR enzymes prior to cycling, takes typically up to 15 minutes of incubation at 95°C. This can reduce enzyme activity and result in lower yield of PCR product. HotMaster *Taq* does not require an initial heat-activation step and can efficiently amplify short fragments as well as fragments up to several kb with high specificity. This combination of high efficiency and high specificity guarantees detection of extremely low levels of target DNA. Even in the presence of high levels of non-template DNA, HotMaster *Taq* can amplify less than 10 target DNA molecules.

5 PRIME HotMasterMix



- ⇒ Optimal results highly specific amplification
- ⇒ Minimal handling ready-to-use mastermix format
- ⇒ Convenience – storage at +4°C eliminates freeze-thaw cycles

Highly specific amplification with minimal handling

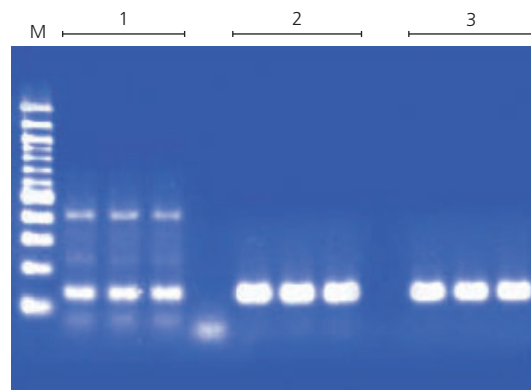
5 PRIME HotMasterMix is a ready-to-use reagent mix that offers high reproducibility when processing large numbers of samples. HotMaster *Taq* DNA polymerase, an integral component of the mastermix, is designed to reduce or eliminate any non-specific products that may result from mispriming during PCR and also features the self-adjusting Mg²⁺ buffer technology.

5 PRIME HotMasterMix is a ready-to-use PCR mix. Only primer and template need to be added to the 2.5x concentrate, decreasing the number of time-consuming pipetting steps. This format not only reduces the likelihood of errors and the risk of contamination, but it also increases precision and sample throughput.

The HotMasterMix does not need to be stored frozen, eliminating the time-consuming thawing process and the resulting reduction in performance. The 5 PRIME HotMasterMix is a 2.5-fold concentrate, resulting in the following final concentrations for a 50 µl PCR reaction:

- 1 U *Taq* DNA Polymerase
- 45 mM KCl
- 2.5 mM Mg²⁺
- 200 µM of each dNTP

Comparison of 5 PRIME *Taq* Enzymes



Amplification of a 131 bp TNF fragment using different 5 PRIME *Taq* enzymes.

- M 100 bp Marker
- 1 Standard *Taq*
- 2 HotMaster *Taq*
- 3 HotMasterMix

Ordering Information

Ref. No.	Product Name	Volume	Quantity
2200300	HotMaster <i>Taq</i> DNA Polymerase - 100 U	20 µl	100 U
2200310	HotMaster <i>Taq</i> DNA Polymerase - 250 U	50 µl	250 U
2200320	HotMaster <i>Taq</i> DNA Polymerase - 1000 U	4 x 50 µl	1000 U
2200330	HotMaster <i>Taq</i> DNA Polymerase - 5000 U	20 x 50 µl	5000 U
2200400	HotMasterMix – 100 Rxn	2 x 1 ml	100 Rxn* = 100 U
2200410	HotMasterMix – 1000 Rxn	20 x 1 ml	1000 Rxn* = 1000 U

*Rxn: 1 reaction of 50 µl

For a complete listing of 5 PRIME PCR reagents and kits please refer to our website www.5PRIME.com.

For a list of international distributors, visit our website at www.5PRIME.com.

For further information, please send an email to info@5PRIME.com.

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