

Increased PCR amplification specificity using illustra Hot Start Master Mix

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We highlight the hot start capability of illustra™ Hot Start Master Mix, providing evidence of its polymerase-blocking ability and elimination of primer-dimer formation.

Introduction

Hot start PCR is a method developed to eliminate nonspecific amplification and limit primer-dimer formation during PCR. Both of these conditions can generate spurious bands, and background smears, and reduce the overall efficiency and yield of PCR. Traditional hot start methods rely on maintaining the polymerase in an inactive state through monoclonal antibodies directed against the polymerase, or by chemical modification of the enzyme itself (1, 2).

Hot Start Master Mix employs a novel and highly efficient method, in which an activator protein sequesters the primers away from the polymerase, making them unavailable for nonspecific priming during reaction setup. Unlike chemically modified hot start polymerases, which can require extensive precycling heat treatment, the DNA-binding activity of the Hot Start Activator protein employed here is readily inactivated during a much gentler precycling step. This characteristic lessens the chances for template DNA damage prior to the cycling steps, which is important when using small quantities of starting material or if amplification of long fragments is required. The result is a more efficient and reliable amplification of the target amplicons.

Methods

Polymerase chain reactions were set up according to the manufacturer's protocols (3).

Results and discussion

Hot Start Master Mix blocks polymerase activity

The ability to maintain a DNA polymerase in an inactive state underpins all hot start PCR systems. This goal is normally achieved via antibody-mediated inhibition or chemical modification of the polymerase. Hot Start Master Mix uses a novel method where the oligonucleotides are sequestered from the polymerase. Thus, the activity of the polymerase is blocked by the removal of one of its substrates. Polymerase-blocking

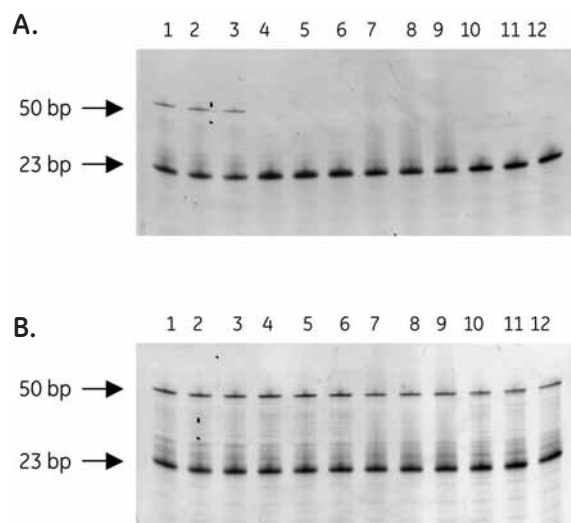


Fig 1. Comparison of Hot Start Master Mix to other commercially available hot start systems in a polymerase blocking assay. Reactions in lanes 1–3 used master mix, lanes 4–6 used Hot Start Master Mix, lanes 7–9 used antibody-inhibited polymerase master mix and lanes 10–12 used a chemically inhibited polymerase master mix. Reactions in Panel A were incubated for 4 h at 25 °C. Reactions in Panel B were heat-treated at 95 °C for 2 min (lanes 4–9) or at 95 °C for 15 min (lanes 10–12) to stop the respective inhibitory mechanisms of the master mixes. All reactions were resolved on a 15% polyacrylamide TBE-Urea denaturing gel and quantitated using ImageQuant™ software.

ability was demonstrated by using an assay that compared the polymerase activity of Hot Start Master Mix to that of a master mix in which the Hot Start Activator protein was heat-inactivated by incubation at 95 °C for 2 min (Fig 1).

A marked increase in the amount of product (50-bp fragment) formed in all the hot start-capable reactions suggests that the polymerase was activated. The results show that Hot Start Master Mix blocked polymerase activity to 99.7% while the monoclonal antibody-inhibited (Invitrogen) and chemically modified (Qiagen) polymerases blocked activity to 97% and 99.4%, respectively. This demonstrates that in this test, Hot Start Master Mix blocks polymerase activity by primer sequestration to a higher degree than other commercially available hot start master mixes.

Hot Start Master Mix inhibits primer-dimer formation

PCR amplification using primers with homology to one another is often unavoidable due to the sequence constraints of the region to be amplified. This situation often results in the formation of primer-dimers, in which the primers anneal and are extended by active polymerase in the presence of dNTPs during reaction setup. This renders the primers unusable for subsequent cycling reactions because of the loss of specificity for the intended target resulting in a decrease in the yield of specific PCR product.

To demonstrate the ability of Hot Start Master Mix to inhibit primer-dimer formation, forward and reverse primers specific for the amplification of either a 303-bp fragment from the human numb locus or a 1114-bp fragment from the human p53 locus were designed and utilized in the primer-dimer assays (Fig 2). Equal volumes of each reaction were resolved on a 1.5% agarose TAE gel stained with ethidium bromide and the average overall yield for a 25- μ l reaction of the specific, amplified product for each master mix was determined with an Agilent Bioanalyzer.

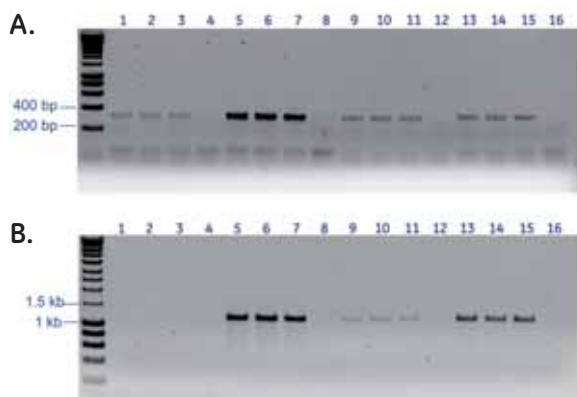


Fig 2. Primer-dimer inhibition. In all the reactions, 1 ng of human genomic DNA was used as template and 0.2 μ M each of forward and reverse primers specific for the human numb locus (A) or human p53 locus (B), which amplified fragments of 303-bp and 1114-bp, respectively. Prior to cycling, all the reactions were held at 25 $^{\circ}$ C for 60 min. For Panels A and B, lanes 1–4 used non-hot start PCR master mix reactions, lanes 5–8 used Hot Start Master Mix, lanes 9–12 used antibody-inhibited polymerase master mix, and lanes 13–16 used chemically inhibited polymerase master mix. Lanes 4, 8, 12, and 16 represent control reactions without templates. Products were resolved on a 1.5% agarose TAE gel stained with ethidium bromide.

Figure 2B depicts the results of the primer-dimer inhibition assay for the 1114-bp p53 locus DNA fragment. As with the numb locus primers, amplification of the 1114-bp p53 fragment was weak and barely visible on an agarose gel (Fig 2B, lanes 1–3). Quantitation was unable to detect a significant peak, indicating a complete failure of amplification.

In a sharp contrast, reactions using the Hot Start Master Mix (Fig 2B, lanes 5–7) produced an average yield of 102 ng. Comparatively, this represents a greater than 13-fold more product than the antibody-inhibited polymerase master mix, whose overall average yield was only 7.4 ng. The chemically inhibited polymerase master mix performed slightly better than the antibody-inhibited polymerase with an overall average yield of 48 ng but still two-fold less than reactions using Hot Start Master Mix.

Conclusions

- > Hot Start Master Mix blocks polymerase activity using primer sequestration to a level comparable to that seen for direct inhibition using anti-*Taq* monoclonal antibody or chemical modification.
- > Hot Start Master Mix more effectively inhibits the formation of primer-dimers during PCR than other commercially available products, thus maximizing target amplification efficiency.
- > Hot Start Master Mix does not interfere with downstream applications such as restriction digestion and direct sequencing of the amplified PCR product (3). Please refer to the application note "Increased PCR amplification specificity using Illustra Hot Start Master Mix" (28-4073-38).

For more information, visit www.gehealthcare.com/hotstartpcr

References

1. Kellogg, D.E. *et al.* TaqStart Antibody: "hot start" PCR facilitated by a neutralizing monoclonal antibody directed against *Taq* DNA polymerase. *BioTechniques* **16**, 1134–1137 (1994).
2. Moretti, T. *et al.* Enhancement of PCR amplification yield and specificity using AmpliTaq Gold DNA polymerase. *BioTechniques* **25**, 716–22 (1998).
3. Application note: Increased polymerase chain reaction (PCR) amplification specificity using illustra Hot Start Master Mix, GE Healthcare, 28-4073-38, Edition AA (2006).

Ordering information

Product	Pack size	Code number
illustra Hot Start Master Mix	100 reactions	25-1500-01