illustra Hot Start Mix RTG

Description

Hot Start Mix RTG is a premixed, predispensed, ambient-temperature-stable formulation that can effectively reduce nonspecific priming and primer-dimer formation during PCR. The system is based on a novel PCR method that uses a Hot Start Activator protein to sequester primers prior to PCR, thereby making them unavailable for nonspecific priming during the reaction preparation. The system requires less vigorous PCR activation conditions than most other commercially available hot start PCR kits.

The advantages of this new Ready-To-Go (RTG) format over other hot start mixes include increased reproducibility across reactions and a more convenient reaction setup. You can expect consistent and reliable PCR results with Hot Start Mix RTG because we have minimized pipetting steps and reduced the potential for errors and contamination.

The only additional reagents required are water, primers, and template DNA. Additional $MgCl_2$ can be easily added allowing users to customize this reagent to their specific needs. The beads are provided predispensed into either 0.2-ml or 0.5-ml PCR tubes. The 0.2-ml tubes are supplied as individual strips of eight tubes to be easily removed. This flexibility allows use of multiple strips of eight or individual 0.2-ml tubes.

Benefits

Hot Start Mix RTG offers:

- **Reproducibility:** Preformulated and predispensed Hot Start Mix RTG beads minimize pipetting steps thus reducing the potential for pipetting errors and contamination of subsequent reactions.
- **Convenience:** Preoptimized and predispensed into PCR tubes, single-dose, ambient temperature stable beads make performing hot start PCR as simple as adding template DNA solution and primer to the tube and cycling the reaction.
- **Specificity:** Reduced primer-dimer formation and non-specific priming leads to increased amplification specificity and efficiency.
- **Reduced risk of contamination:** Use of PuReTaq™ DNA Polymerase and other high-purity reagents ensure the lowest possible levels of contaminating DNA in each bead.
- **Ambient-temperature stability:** Hot Start Mix RTG beads can be shipped and stored at room temperature.

Applications

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 increased PCR specificity of Hot Start Mix RTG, amplifications of a 1018-bp fragment of a human gene were performed with a precycling incubation of 25°C for 1 h to allow primer-dimer formation and nonspecific amplification.

The average amplification yield of the 1018-bp fragment for the Hot Start Mix RTG (lanes 1 and 2) was 295 ng, while the average yield for AmpliTaq™ DNA Polymerase was 40 ng. The non-hot start reactions (lanes 4 and 5) showed nonspecific amplification and lower yield of the specific fragment.

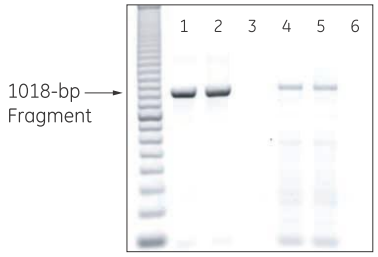

Fig 1. Amplification of a 1018-bp fragment of a human gene. In all the reactions, 2 ng of human genomic DNA and 10 pmol each of forward and reverse primers specific for the 1018-bp fragment were used. Lanes 1 and 2 contain Hot Start Mix RTG; 2 and 4, AmpliTaq DNA polymerase. No-template control reactions for each sample are in lanes 3 and 6. All the products were resolved on a 1.5% agarose TAE gel stained with ethidium bromide.

Hot Start Mix RTG beads give reproducible results when used in real-time PCR assays. Figure 2 shows the reproducibility of a Lambda DNA titration with Hot Start Mix RTG beads in an intercalating dye real-time PCR assay. These results show that you can rely on the bead-to-bead reproducibility of the system.

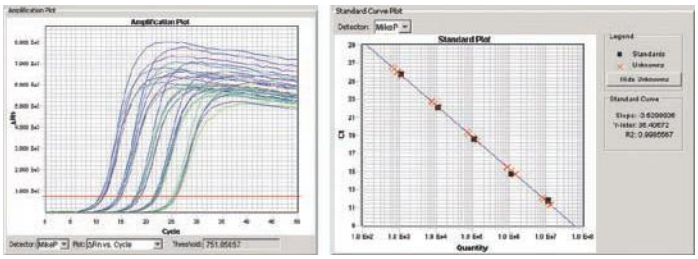

Fig 2. Bead-to-bead reproducibility of a Lambda DNA titration from 1.0 x 10^3 to 1.0 x 10^7 copies in an intercalating dye real-time PCR assay. Ten replicates of each template concentration were cycled on the ABI 7900HT Fast Real-time PCR System. The standard curve resulted in a R^2 value of greater than 0.99.

Components

The Hot Start Mix RTG formulation is composed of recombinant PuReTaq DNA polymerase, dNTPs, buffer, stabilizers, and Hot Start Activator protein. Additional $MgCl_2$ can be added to optimize the PCR conditions.

Quality control

Hot Start Mix RTG was functionally tested:

- To demonstrate that polymerase activity is blocked by 90% or greater when incubated for 4 h at 25°C in the presence of DNA polymerase, dNTPs, and polymerase substrate compared to reactions in which the Hot Start Activator protein had been inactivated by heat treatment.
- To ensure that Hot Start Mix RTG is free of contaminating prokaryotic and eukaryotic genomic DNA. Real time PCR assays were performed and the levels of all these potential contaminants were found to be negligible.

Storage

Store Hot Start Mix RTG at ambient temperature in pouch with desiccant.

Ordering information

Hot Start Mix RTG 0.5-ml tubes, 100 reactions	28-9006-46
Hot Start Mix RTG 0.2-ml tubes, 96 reactions	28-9006-53
Hot Start Mix RTG 0.2-ml tubes, 5 x 96 reactions	28-9006-54

Related products

FideliTaq™ DNA Polymerase	E71180
FideliTaq PCR Master Mix (2X)	E71182
FideliTaq PCR Master Mix Plus	E71183
Taq DNA Polymerase	see catalog
PuReTaq Ready-To-Go™ PCR Beads	see catalog
Ready-To-Go RT-PCR Beads	see catalog
FideliTaq RT-PCR Master Mix (2X)	E71185
RT-PCR Master Mix (2X)	E78370
dNTPs for PCR and Long PCR, Pre-Mixed	see catalog
ExoSAP-IT™	see catalog
GFX™ PCR DNA and Gel Band Purification Kit, 100 purifications	27-9602-01
GFX 96 PCR Purification Kit, 10 x 96-well plates	25-6902-02
MicroSpin™ S-400 HR Columns	27-5140-01

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