USB® HotStart-IT® FideliTaq™ PCR Master Mix (2X)

- High-specificity and high-sensitivity PCR amplification
- Extremely long PCR amplification
- PCR-mediated cloning
- High throughput PCR

USB HotStart-IT FideliTaq Master Mix combines a novel hot start method designed and developed at Affymetrix with the long and accurate amplification properties of FideliTaq DNA Polymerase. The hot start method, called primer sequestration, uses a binding protein to reduce or eliminate non-specific primer-extension products which may be generated at lower temperatures during assembly of PCR reactions. Following the initial denaturation step during PCR, the binding protein is inactivated and the primers are free to participate in the amplification reaction. This novel hot start method enhances many complex PCR reactions by increasing specificity and yield (Fig. 1) as well as sensitivity (Fig. 2).

HotStart-IT FideliTaq Master Mix combines HotStart-IT FideliTaq DNA Polymerase and Ultrapure nucleotides in a proprietary reaction buffer. Simply add template, primers, and water and the reactions are ready for cycling. The mix increases amplification fidelity up to 6-fold over Taq DNA Polymerase alone and generates much longer PCR products\(^{1-6}\) (Fig. 3). PCR products have ends which are compatible with either blunt-end or TA cloning procedures\(^{9}\) with A-tailed ends favored over blunt ends in an approximately 3 to 1 ratio.

**Novel hot start technology**

The mix does not use Taq antibody which eliminates potential mammalian-source DNA contamination. Also, since the polymerase is not chemically-inactivated, no extensive heat-activation step is necessary which reduces damage to precious DNA samples.

**Convenient**

Save time and reduce potential contamination errors by eliminating several pipetting steps. For a 50 μl reaction, simply add 25 μl of HotStart-IT FideliTaq Master Mix to primers, DNA template and PCR-Qualified H₂O. Reactions can be tailored from 10 μl to 100 μl volumes.

Room temperature reaction assembly is possible because of the hot start feature.

**Higher specificity and sensitivity**

Minimize amplification of non-specific products and primer-dimers (Fig. 1). Amplify PCR products with low background and from low-copy targets, essential for demanding genomic and cDNA applications with limited sample material (Fig. 2).

**Fig. 1. Increased specificity**

[Increased specificity of the HotStart-IT FideliTaq PCR Master Mix and stability. A 306 bp fragment of the single-copy numb gene was amplified from 1 ng of human genomic DNA with and without USB HotStart-IT technology. The primers in this assay were designed with 3 bases of overlap at their 3'-ends to favor primer-dimer formation during reaction set-up at room temperature. Results demonstrate a shift from mainly primer-dimers to the desired product when HotStart-IT is used. Also, the mix is extremely stable as no loss in performance was observed following 10 freeze-thaw cycles (FTs) relative to a mix stored at -20°C.]

**Fig. 2. Sensitivity**

[Sensitivity of the HotStart-IT FideliTaq PCR Master Mix. A 455 bp fragment of the single-copy numb gene was amplified from the indicated amounts of human genomic DNA. The master mix is extremely sensitive as amplification can be achieved from approximately one human cell.]
**High fidelity**
Obtain up to 6 fold higher fidelity than Taq DNA Polymerase, ideal for cloning and microarray applications.

**Increase product size and yield**
Amplify very long PCR products from complex DNA templates with little or no optimization (Fig. 3). For PCR products greater than 2 kb, yields are greatly increased.

**Stable**
Repeated freeze-thaw cycles have no observed effect on performance (Figs. 1 and 3).

**Functional tests:**
PCR with HotStart-IT FideliTaq Master Mix shifts production of primer-dimers to a specific target of 306 bp from 1 ng of human genomic DNA relative to FideliTaq Master Mix.

PCR with HotStart-IT FideliTaq Master Mix generates a 20.7 kb product from lambda DNA.

**HotStart-IT FideliTaq Master Mix Formulation (2X):**
HotStart-IT FideliTaq Master Mix combines USB FideliTaq DNA Polymerase with a recombinant hot start protein in a unique buffer formulation. Magnesium and nucleotide concentrations are 3 mM and 0.4 mM each, respectively.

**Components:**
HotStart-IT FideliTaq Master Mix (2X)
- 25 reactions (625 µl)
- 100 reactions (4 x 625 µl)
- 500 reactions (12.5 ml)

**Brief protocol**

**Shipping and storage:**
Shipped on dry ice. Store at -20°C. Mix well prior to use.

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**HotStart-IT FideliTaq PCR Master Mix (2X)**

<table>
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**References:**