

USB® HotStart-IT® Taq Master Mix (2X)

- Minimizes amplification of non-specific products and primer-dimers (Fig. 1).
- High specificity and sensitivity
- Convenient, ready-to-use mix which significantly reduces time-consuming optimization.
- Room temperature reaction set-up
- Unlike chemically-modified Taq, no extensive heating step is necessary which may damage precious samples.

USB HotStart-IT Taq Master Mix uses a novel hot start method designed and developed at Affymetrix called primer-sequestration. In general, hot start PCR methods reduce or eliminate non-specific primer-extension products formed at lower temperatures during assembly of PCR reactions. At these less stringent annealing temperatures, primers may bind non-specifically, which often leads to unwanted amplification products and primer-dimers. In order to resolve this problem, we have combined high-quality USB Taq DNA Polymerase with a recombinant protein which binds and sequesters primers at lower temperatures making them unavailable for use by Taq DNA Polymerase. This primer-sequestration technique effectively blocks DNA synthesis from mis-priming events at lower temperatures. Following the initial denaturation step during PCR, the 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 both specificity and yield.

HotStart-IT Taq Master Mix (2X) combines high-quality USB recombinant Taq DNA Polymerase, a recombinant hot start protein, and USB Ultrapure nucleotides in a proprietary reaction buffer. This ready-to-use mix provides robust and reliable performance for demanding PCR applications in which high specificity and high sensitivity are desired. Since the mix is pre-formulated, experimental variability is significantly reduced.

Convenience:

The pre-mixed formulation saves time and reduces potential contamination errors by eliminating several pipetting steps. For a 50 µl reaction, simply add 25 µl of HotStart-IT Taq Master Mix to primers, DNA template and PCR-Qualified H₂O. Reactions can be easily performed in 10 µl, 25 µl, 50 µl or 100 µl volumes.

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

Higher specificity and sensitivity:

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

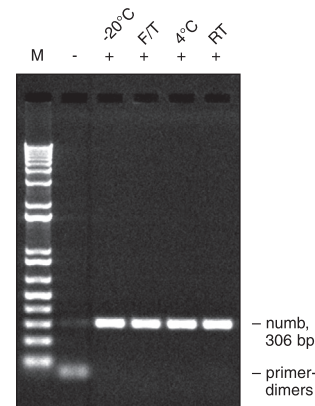
Stable performance:

The mix withstands repeated freeze-thaw cycles and 4°C storage for extended periods of time with no observed decrease in performance (Fig. 1).

Novel hot start technology:

The mix contains no Taq antibody and therefore eliminates the risk of mammalian-source contamination. Also, since the polymerase is not chemically-inactivated, there is no extensive heating step necessary which reduces the chance of damaging precious DNA samples from heat-induced depurination.

Fig. 1. Increased specificity and stability



Increased specificity and stability of HotStart-IT® Taq Master Mix.

Specificity: The single-copy numb gene was amplified from 1 ng of human genomic DNA with standard Taq Master Mix (-) or with HotStart-IT Taq Master Mix (+). Primers were designed with a 3 bp 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 Taq Master Mix is used. **Stability:** The Master Mix shows no loss in performance following 15 freeze-thaw cycles (F/T), 4°C storage for one month (4°C), or room temperature storage for one month (RT) compared to a reference mix stored at -20°C.

Polymerase blocking assay:

The assay compares the polymerase activity of HotStart-IT Taq Master Mix [PN 71196] relative to Taq Master Mix [PN 71162]. The reaction mixtures contain 1X Master Mix and 2 pmol of overlapping, extendable oligonucleotides in a 25 µl reaction volume. Following incubation at 25°C for 4 hours, HotStart-IT Taq Master Mix blocks at least 90% of the activity relative to Taq Master Mix without hot start capability (Fig. 2).

Tested User Friendly™ functional test:

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

HotStart-IT Taq Master Mix formulation (2X):

HotStart-IT Taq Master Mix combines USB Taq 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. Brief protocol card is also provided.

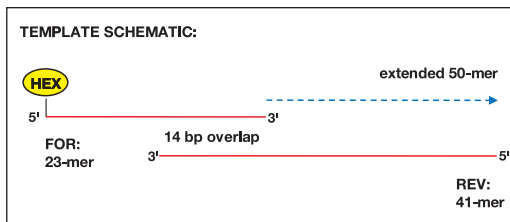
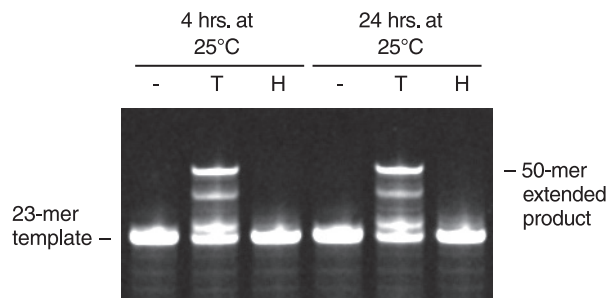
Shipping and storage:

Shipped on dry ice. Store at -20°C. If desired, after initial thawing, the product may be stored at 4°C. Mix well prior to use.

HotStart-IT Taq Master Mix (2X)

Product code	Pack size
71196	25 reactions
	100 reactions
	500 reactions

Fig. 2. Activity is blocked at room temperature.



Two overlapping and extendable oligonucleotides were incubated in a mock PCR reaction at 25°C for either 4 or 24 hours with standard Taq Master Mix (T), HotStart-IT Taq Master Mix (H), or no polymerase (-). Following incubation, reactions were separated on a 15% denaturing gel with urea. Results demonstrate that full-length extension of the HEX-labeled 23-mer to a 50-mer was completely blocked by HotStart-IT Taq Master Mix at room temperature while some extension occurred with the standard Taq Master Mix.

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