

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



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

USB HotStart-IT Fidelity PCR Master Mix combines a novel hot start method designed and developed at Affymetrix with the long and accurate amplification properties of Fidelity 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 Fidelity PCR Master Mix combines HotStart-IT Fidelity 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<sup>(1-4)</sup> (Fig. 3). PCR products have ends which are compatible with either blunt-end or TA cloning procedures<sup>(5)</sup> 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

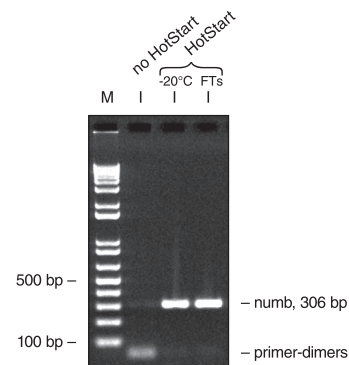
Save time and reduce potential contamination errors by eliminating several pipetting steps. For a 50 µl reaction, simply add 25 µl of HotStart-IT Fidelity PCR Master Mix to primers, DNA template and PCR-Qualified H<sub>2</sub>O. Reactions can be tailored from 10 µl to 100 µl volumes.

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

## Higher specificity and sensitivity

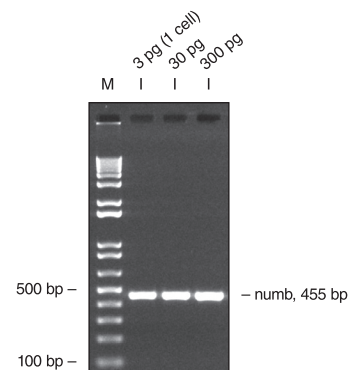
Minimize amplification of non-specific products and primer-dimers (Fig. 1). Amplify PCR products with low background and from low-copy targets, essential for demanding genomic and cDNA applications with limited sample material (Fig. 2).

Fig. 1. Increased specificity



Increased specificity of the HotStart-IT Fidelity 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 Fidelity 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).

### Tested User Friendly™ functional tests:

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

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

### HotStart-IT FidelityTaq Master Mix Formulation (2X):

HotStart-IT FidelityTaq Master Mix combines USB FidelityTaq 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 FidelityTaq 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.

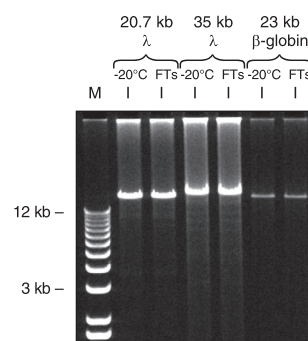
### HotStart-IT FidelityTaq PCR Master Mix (2X)

Product code	Pack size
71156	25 reactions
	100 reactions
	500 reactions

### References:

1. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 2216-2220.
2. Cheng, S., Focklet, C., Barnes, W. M., and Higuchi, R. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 5695-5699.
3. Barnes, W. M. (1992) *Gene* **112**, 29-35.
4. Cline, J., Braman, J. C., and Hogrefe, H. H. (1996) *Nucl. Acids Res.* **24**, 3546-3551.
5. Magnuson, V. L., Ally, D. S., Nylund, S. J., Karanjawala, Z. E., Rayman, J. B., Knapp, J. I., Lowe, A. L., Ghosh, S., and Collins, F. S. (1996) *BioTechniques* **4**, 700-709.

Fig. 3. Extreme amplicon length



Extreme amplicon length using HotStart-IT FidelityTaq PCR Master Mix and stability. Two long PCR products of 20.7 kb and 35 kb were amplified from lambda DNA using 1 ng and 10 ng of DNA respectively. A 23 kb fragment of the  $\beta$ -globin gene was amplified from 100 ng of human genomic DNA. A denaturation temperature of 95°C was used for lambda targets and 92°C was used for the human target. Note that 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.

For research use only. Not for use in diagnostic procedures.

Affymetrix, USB and HotStart-IT are registered trademarks of Affymetrix, Inc. Tested User Friendly and FidelityTaq are trademarks of Affymetrix, Inc. HotStart-IT Taq DNA Polymerase—Methods for using this product may be covered by US Patent No. 7,700,281. FidelityTaq DNA Polymerase technology is licensed under US Patent No. 5,436,149 owned by TaKaRa Bio Inc. Taq DNA Polymerase—sold under licensing arrangements with Applied Biosystems. Purchase is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler.

### Affymetrix, Inc.

#### USB® Products

26111 Miles Road  
Cleveland Ohio 44128

Tel: 800-321-9322 | 216-765-5000

Fax: 800-535-0898 | 216-464-5075

USBcustomerserv@affymetrix.com

[usb.affymetrix.com](http://usb.affymetrix.com)

### Affymetrix UK Ltd.

#### USB® Products

Voyager, Mercury Park,  
Wycombe Lane, Wooburn Green,

High Wycombe HP10 0HH, United Kingdom

Tel: +44 (0)1628 55 2600

Fax: +44 (0)1628 55 2675

USBcustomerserveurope@affymetrix.com

### Affymetrix Pte Ltd

#### USB® Products

7 Gul Circle, #2M-01

Keppel Logistics Building

Singapore 629563

Tel: +65 63957301

Fax: +65 63957300

USBcustomerserv@affymetrix.com