

# USB® HotStart-IT® FidelityTaq™ DNA Polymerase



- Room temperature reaction set-up
- Minimizes amplification of non-specific products and primer-dimers (Fig. 1)
- High specificity and sensitivity (Fig. 2)
- Generates long PCR amplicons (Fig. 3)
- Up to 6-fold higher fidelity than Taq DNA Polymerase
- Does not use antibodies, eliminating potential mammalian-source DNA contamination
- No extensive heating step is necessary which may damage precious samples, like with chemically-modified Taq

**Storage buffer:**

20 mM Tris-HCl (pH 8.5), 1 mM DTT, 0.1 mM EDTA, 200 mM KCl, 50% glycerol, and stabilizers.

USB HotStart-IT FidelityTaq DNA Polymerase combines a novel hot start method designed and developed at Affymetrix with the long and accurate amplification properties of FidelityTaq 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).

FidelityTaq DNA Polymerase combines USB Taq DNA Polymerase with a high-fidelity, proofreading polymerase. This enzyme blend has the 5'→3' exonuclease activity of Taq DNA Polymerase as well as the 3'→5' exonuclease activity of the proofreading enzyme. FidelityTaq DNA Polymerase increases fidelity up to 6-fold over Taq DNA Polymerase alone and allows for amplification of longer products<sup>(1-4)</sup> (Fig. 3). The enzyme blend generates PCR products whose ends 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.

**Applications:**

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

**Source:**

All proteins are recombinant versions expressed in *E. coli*.

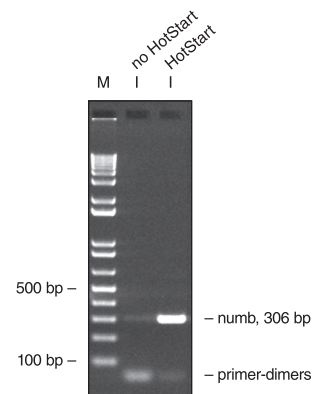
**Properties:**

Activator: Mg<sup>2+</sup>

**Purity:**

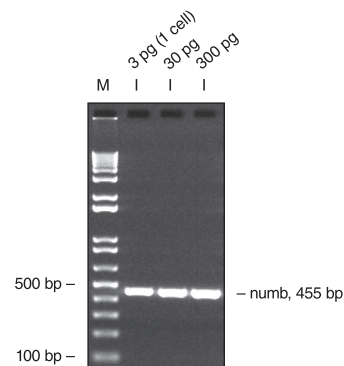
Free from detectable non-specific nucleases.

**Fig. 1. Increased specificity**



Increased specificity of HotStart-IT FidelityTaq DNA Polymerase. 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.

**Fig. 2. Sensitivity**



Sensitivity of HotStart-IT FidelityTaq DNA Polymerase. A 455 bp fragment of the single-copy numb gene was amplified from the indicated amounts of human genomic DNA. The polymerase is extremely sensitive as amplification can be achieved from approximately one human cell.

### Unit definition:

One unit incorporates 10 nmol of total nucleotides into acid-insoluble material in 30 minutes at 74°C in a total volume of 50 µl<sup>(6-7)</sup>.

### Concentration:

2.5 units/µl

### Assay conditions:

The reaction mixture contains 25 mM TAPS, pH 9.3 (at 25°C), 50 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM 2-mercaptoethanol, 200 µM dNTPs, 250 µg/ml activated salmon sperm DNA and Taq DNA Polymerase. Following incubation at 74°C for 10 minutes, acid-insoluble material is determined.

### Tested User Friendly™ functional tests:

PCR with HotStart-IT FidelityTaq DNA Polymerase shifts production of primer-dimers to a specific target of 306 bp from 1 ng of human genomic DNA relative to FidelityTaq DNA Polymerase. PCR with HotStart-IT FidelityTaq DNA Polymerase generates a 20.7 kb product from lambda DNA.

### Functionally Tested 10X PCR Reaction Buffer (included, PN 71165):

100 mM Tris-HCl (pH 8.6), 500 mM KCl, 15 mM MgCl<sub>2</sub>

### Functionally Tested MgCl<sub>2</sub> (included, PN 71167):

25 mM solution

### Shipping and storage:

Shipped on dry ice. Store at -20°C.

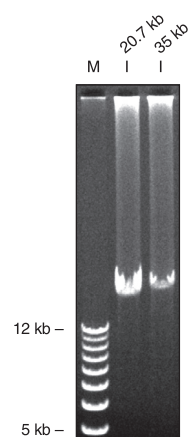
### HotStart-IT FidelityTaq DNA Polymerase

Product code	Pack size
71155	50 units
	250 units
	1,000 units
	5,000 units

### References:

1. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 2216-2220.
2. Cheng, S., Fockler, 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.
6. Innis, M. A., Myambo, K. B., Gelfand, D. H. and Brow, M. A. (1988) *Proc. Natl. Acad. Sci.* **85**, 9436-9440.
7. Lawyer, F. C., Stoffel, S., Saiki, R. K., Myambo, K., Drummond, R. and Gelfand, D. H. (1989) *J. Biol. Chem.* **264**, 6427-6437.

Fig. 3. Extreme amplicon length



Extreme amplicon length using HotStart-IT FidelityTaq DNA Polymerase. 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. Cycling conditions consisted of a short initial denaturation phase at 95°C for 20 seconds and 25 cycles of 95°C for 5 seconds and 68°C for 20 minutes.

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