Affymetrix® | USB® Products

PCR Tools

USB® HotStart-IT® Taq DNA Polymerase

- Minimizes amplification of non-specific products and primer-dimers (Fig. 1).
- High specificity and sensitivity
- Room temperature reaction set-up
- Unlike chemically-modified Taq, no extensive heating step is necessary which may damage precious samples.

USB HotStart-IT Taq DNA Polymerase has been designed for PCR applications that demand high specificity and sensitivity. HotStart-IT Taq is thoroughly tested for purity and performance and is supplied with a 10X PCR Reaction Buffer and a separate tube of 25 mM $MgCl_2$.

HotStart-IT Taq DNA Polymerase 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.

Source:

E. coli strain expressing a clone of Taq DNA Polymerase from *Thermus aquaticus*[1-3]. The hot start component is a recombinant protein also expressed in *E. coli.*

Concentration:

1.25 units/µl

Properties:

Activator: Mg^{2+}

USB Taq DNA Polymerase is a highly processive 5′→3′ polymerase and has 5′→3′ exonuclease activity. Suitable for TaqMan® assays.

Purity:

Free from detectable non-specific nucleases.

Storage buffer:

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

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.

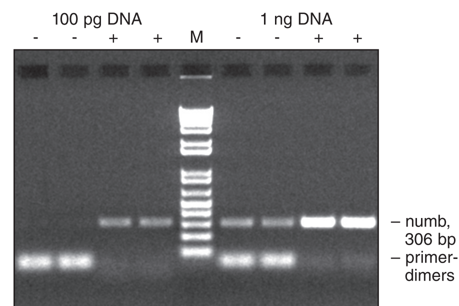
Assay conditions:

The reaction mixture (50 µl) contains 25 mM TAPS, pH 9.3 (at 25°C), 50 mM KCl, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 200 µM dNTPs, 250 µg/ml activated salmon sperm DNA and cloned Taq DNA Polymerase. After incubation at 74°C for 10 minutes, acid-insoluble material is determined.

Polymerase blocking assay:

The assay compares the polymerase activity of HotStart-IT Taq DNA Polymerase relative to Taq DNA Polymerase [PN 71160]. The reaction mixtures contain 0.625 units of polymerase, 1X PCR Reaction Buffer, 0.2 mM each dNTP, and 2 pmol of overlapping, extendable oligonucleotides in a 25 µl reaction volume. Following incubation at 25°C for 4 hours, HotStart-IT Taq DNA Polymerase blocks at least 90% of the activity relative to Taq DNA Polymerase without hot start capability (Fig. 2).

Fig. 1. Increased specificity



Increased specificity of HotStart-IT Taq DNA Polymerase. The single-copy numb gene was amplified from the indicated amounts of human genomic DNA with standard Taq DNA Polymerase (-) or with HotStart-IT Taq DNA Polymerase (+). 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 DNA Polymerase is used.

Tested User Friendly™ functional test:

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

Functionally tested 10X PCR Reaction Buffer [PN 71165 included]:

100 mM Tris-HCl (pH 8.6), 500 mM KCl, 15 mM $MgCl_2$

Functionally tested $MgCl_2$ [PN 71167 included]:

25 mM solution

Applications:

1. High-specificity PCR amplification
2. High-sensitivity PCR amplification
3. TA-vector cloning
4. Amplification prior to *in vitro* transcription

Shipping and storage:

Shipped on dry ice. Store at -20°C.

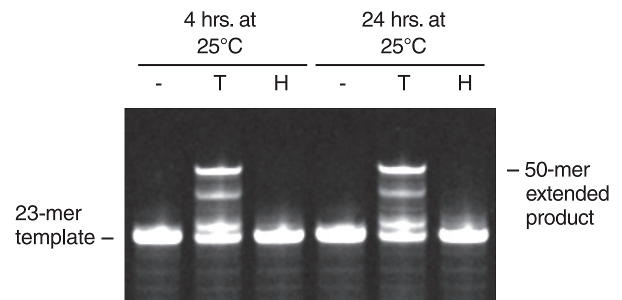
HotStart-IT Taq DNA Polymerase

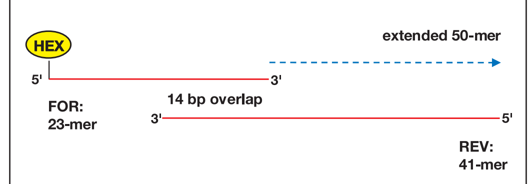
Product code	Pack size
71195	50 units
	250 units
	1,000 units
	5 x 250 units
	5,000 units

References:

1. Innis, M. A., Myambo, K. B., Gelfand, D. H. and Brow, M. A. (1988) *Proc. Natl. Acad. Sci.* **85**, 9436-9440.
2. Lawyer, F. C., Stoffel, S., Saiki, R. K., Myambo, K., Drummond, R. and Gelfand, D. H. (1989) *J. Biol. Chem.* **264**, 6427-6437.
3. Innis, M. A. and Gefland, D. H. (1990) PCR Protocols: A Guide to Methods and Applications, Academic Press.

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 DNA Polymerase (T), HotStart-IT Taq DNA Polymerase (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 DNA Polymerase while some extension occurred with standard Taq DNA Polymerase.

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