

USB® HotStart-IT® Probe qPCR Master Mix (2X)



- **Complete master mix with fluorescent probes**
 - Compatible with TaqMan® probes, Molecular Beacons, FRET probe pairs, etc.
 - Multiple platform capability - includes ROX™ Reference Dye to allow normalization of well-to-well variations
- **Based on novel Hot Start method of primer sequestration**
 - No DNA template damage - no extensive heating step needed to denature hot start component
 - Avoids non-specific products and primer-dimer formation
 - Room temperature reaction set-up
- **Highest sensitivity with broad dynamic range**
 - Detects fewer than 10 target copies
 - Performs over a linear dynamic range of 7 to 8 orders of magnitude with a minimal correlation coefficient = 0.95

USB HotStart-IT Probe qPCR Master Mix uses a novel hot start method designed and developed at Affymetrix called primer sequestration. With this method, a protein binds and sequester primers at lower temperatures making them unavailable for use by Taq DNA Polymerase. Following the initial denaturation step, the protein is inactivated and the primers are released (Fig. 1). HotStart-IT Probe qPCR Master Mix is supplied as a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, MgCl₂, and Ultrapure nucleotides for use in real-time quantitative PCR reactions (qPCR) with fluorescent probes. Simply add DNA

template, primers, probe(s) and water and the reactions are ready for cycling. A separate tube of ROX passive reference dye (for ABI and Stratagene instruments) is included for added convenience.

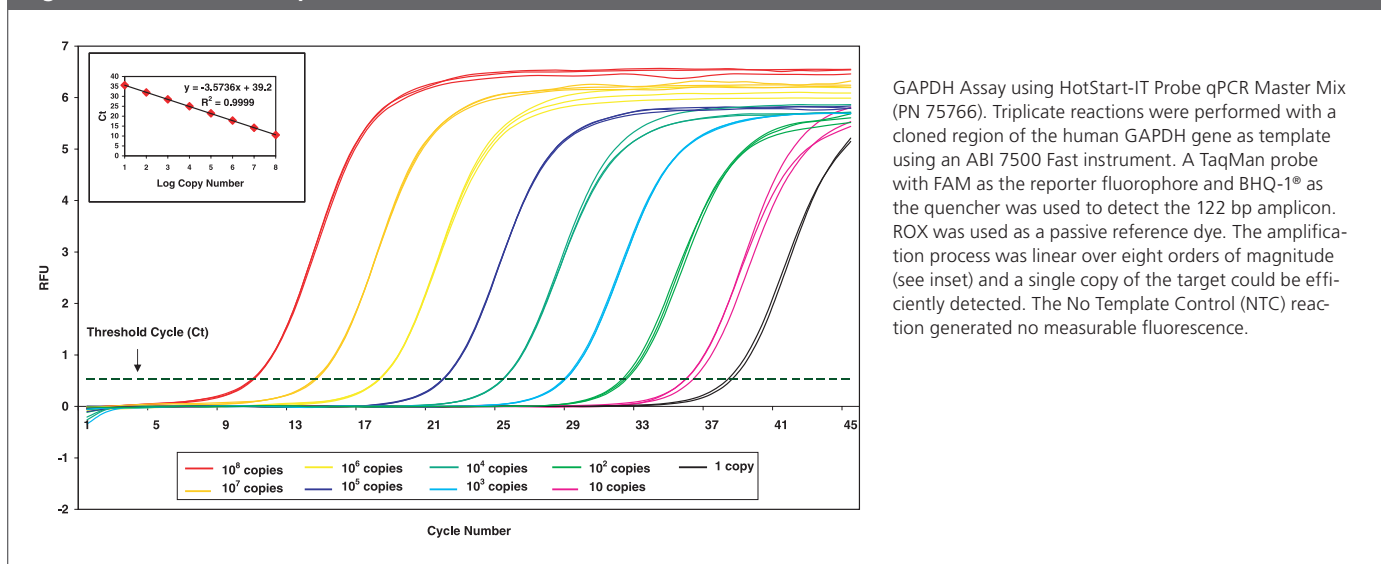
The master mix is formulated for use with fluorescent probes such as TaqMan Probes, Molecular Beacons, and others⁽¹⁻²⁾. Since fluorescent probes are designed to hybridize to the target of interest, detection specificity is greatly increased relative to non-specific dsDNA binding dyes such as SYBR® Green I. The Taq DNA Polymerase used in the master mix has the 5'→3' exonuclease activity necessary for efficient removal of the 5'-fluorophore from the 3'-quencher in TaqMan probes. HotStart-IT Probe qPCR Master Mix has excellent sensitivity as it detects fewer than 10 target copies, performs over a broad, linear dynamic range of 7 to 8 orders of magnitude, and is compatible with a variety of real-time PCR instruments. The mix does not have dUTP in place of dTTP and is incompatible with carry-over contamination prevention methods using Uracil-DNA Glycosylase. For carry-over prevention methods, use USB HotStart-IT Probe qPCR Master Mix with UDG (2X), PN 75764.

Convenient

For a 50 µl reaction, simply add 25 µl of master mix to primers and probe(s), DNA template and PCR-Qualified H₂O. Reactions can be tailored from 20 µl to 100 µl volumes.

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

Fig. 1. Real-time PCR amplification



GAPDH Assay using HotStart-IT Probe qPCR Master Mix (PN 75766). Triplicate reactions were performed with a cloned region of the human GAPDH gene as template using an ABI 7500 Fast instrument. A TaqMan probe with FAM as the reporter fluorophore and BHQ-1® as the quencher was used to detect the 122 bp amplicon. ROX was used as a passive reference dye. The amplification process was linear over eight orders of magnitude (see inset) and a single copy of the target could be efficiently detected. The No Template Control (NTC) reaction generated no measurable fluorescence.

Novel hot start technology

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

Higher specificity, sensitivity and broad dynamic range

The hot start feature minimizes amplification of non-specific products and primer-dimers. The reaction buffer with optimum $MgCl_2$ concentration is specially designed for robust probe hybridization and efficient cleavage of TaqMan probes. PCR products are amplified with low background and from low-copy targets with a linear dynamic range of 7 to 8 orders of magnitude (Fig. 1).

Stable

Repeated freeze-thaw cycles have no observed effect on performance.

Tested User Friendly™ functional tests:

Real-time PCR reactions were performed on an ABI 7500 Fast Instrument using primers and TaqMan probe specific to a 122 bp cloned region of the human GAPDH gene as template. Product specifications require that the correlation coefficient from a linear regression over seven orders of magnitude (10^7 to 10^1 template copies) must be greater than or equal to 0.95.

HotStart-IT Probe qPCR Master Mix (2X):

The mix combines USB HotStart-IT Taq DNA Polymerase (with 5'→3' exonuclease activity), $MgCl_2$, and Ultrapure nucleotides in a unique buffer formulation. Magnesium and nucleotide concentrations are 6 mM and 0.4 mM each, respectively.

Components:

HotStart-IT Probe qPCR Master Mix (2X)
100 reactions (2 x 1.25 ml)
500 reactions (12.5 ml)
25 mM $MgCl_2$
ROX Passive Reference Dye
Brief protocol

Shipping and storage:

Shipped on dry ice. Store at $-20^\circ C$. Mix well prior to use.

HotStart-IT Probe qPCR Master Mix (2X)

Product code	Pack size
75766	100 reactions
	500 reactions

References:

1. Livak, K. J., Flood, S. J., Marmaro, J., Giusti, W., and Deetz, K. (1995) *PCR Methods Appl.* **4**, 357-362.
2. Tyagi, S., and Kramer, F. R. (1996) *Nat. Biotechnol.* **14**, 303-308.

Related products

PrepEase® RNA Spin Kit [78766]

50 preps

PrepEase Plant RNA Spin Kit [78771]

50 preps

PrepEase Tissue & Cells DNA Spin Kit [78861]

50 preps

First-Strand cDNA Synthesis Kit for Real-Time PCR [75780]

50 reactions

AMV Reverse Transcriptase [70041Y,Z]

200 units

1,000 units

M-MLV Reverse Transcriptase [78306]

25,000 units

100,000 units

Water, Nuclease-Free [71786]

10 x 1 ml

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