

PCR Tools

USB® HotStart-IT® Probe One-Step qRT-PCR Master Mix Kit



- **Based on innovative HotStart-IT technology**
 - Proprietary primer sequestration method
 - Increases specificity and prevents primer-dimer formation
 - No DNA template damage – no extensive heating step needed to denature hot start component
- **Convenient**
 - One-step, sequential reaction format
 - Easy analysis of single targets from multiple RNA samples
 - Saves time and reduces potential contamination

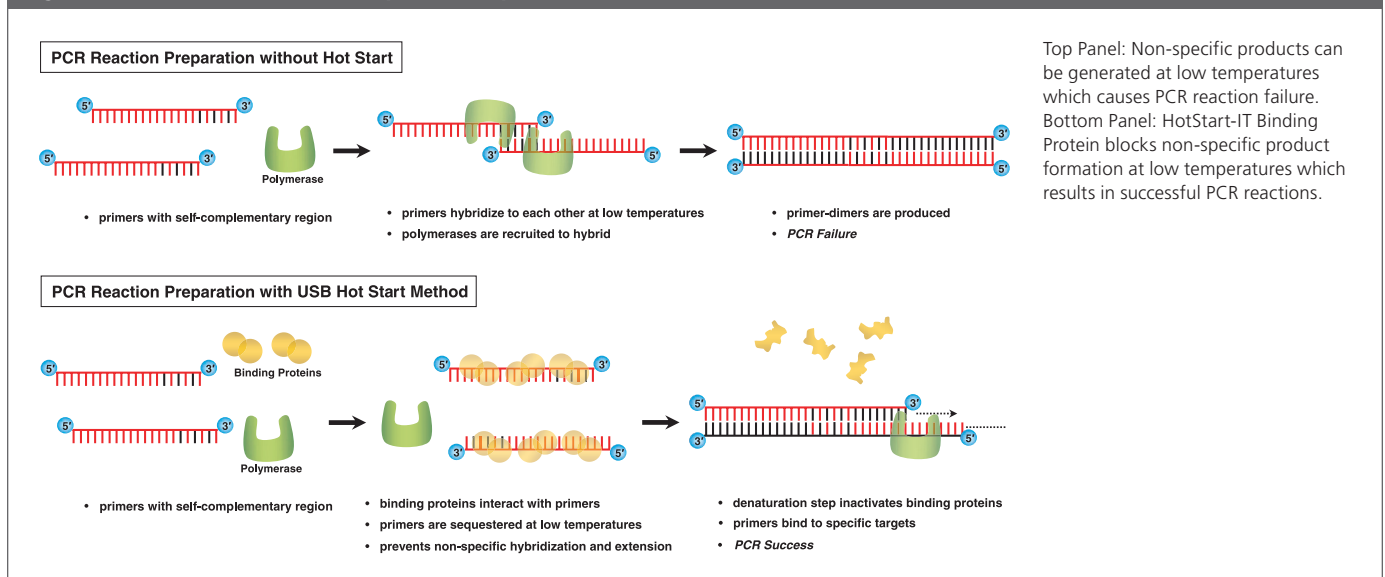
USB HotStart-IT Probe One-Step qRT-PCR Master Mix Kit provides optimal performance and maximum convenience for real-time, quantitative analysis of RNA templates in a single reaction format. The RT-PCR process converts and amplifies single-stranded RNA template yielding double-stranded DNA product. One-Step RT-PCR uses gene specific primers, designed to match RNA/cDNA targets, in a single-tube/plate, one-step reaction. This approach offers tremendous convenience when applied to analysis of single targets from multiple RNA samples. Also, it minimizes the possibility of introducing contaminants into reactions between the RT and PCR steps, since both steps are carried out

sequentially without opening the reaction tubes/plates between the steps⁽¹⁻⁵⁾.

The HotStart-IT Probe One-Step qRT-PCR Master Mix Kit includes M-MLV RT, RNase Inhibitor and a 2X Master Mix containing HotStart-IT Taq DNA Polymerase, MgCl₂, and Ultrapure nucleotides in an optimized reaction buffer for use with fluorescent probes. HotStart-IT Probe qPCR Master Mix uses a novel hot start method designed and developed at Affymetrix called primer sequestration (Fig. 1). With this method, the HotStart-IT protein binds and sequesters primers at lower temperatures making them unavailable for use by Taq DNA Polymerase. Following reverse transcription and the subsequent heat denaturation step, the primer binding protein is inactivated and the primers are released.

This kit is formulated for use with fluorescent probes such as TaqMan® Probes, Molecular Beacons, and others⁽⁶⁻⁷⁾. 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 this master mix has the 5' to 3' exonuclease activity necessary for efficient removal of the 5'-fluorophore from the 3'-quencher in TaqMan probes.

Fig. 1. USB HotStart-IT method: primer sequestration



This kit exhibits excellent sensitivity as it can detect fewer than 10 target copies, performs over a broad, linear dynamic range of 6 to 7 orders of magnitude, and is compatible with most real-time PCR instruments (Fig. 2). Individual kit components have been carefully formulated to obtain optimal activity of M-MLV RT and Taq DNA Polymerase, and efficient probe hybridization to allow highly sensitive and specific detection of RNA transcripts from either total RNA or poly(A)+ mRNA. A separate tube of the passive reference dye, ROX™, is included for added convenience to allow normalization of well-to-well variations.

Tested User Friendly™ functional test:

The HotStart-IT Probe One-Step qRT-PCR Master Mix Kit is a Tested User Friendly product, assuring reliable results. Release specifications for the kit are based on the following functional assay: Real-time qRT-PCR reactions were performed on an ABI 7500 Fast Instrument using primers and TaqMan probe specific to a 122 bp region of the human GAPDH gene and human total RNA as template. Product specifications require that the correlation coefficient from a linear regression over five orders of magnitude (10 pg to 100 ng) must be greater than or equal to 0.95.

HotStart-IT Probe qPCR Master Mix (2X), PN 75766:

This Master Mix is a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, MgCl₂, and Ultrapure nucleotides in an optimized reaction buffer for use with fluorescent probes in real-time, quantitative PCR reactions. Magnesium and nucleotide concentrations are at 6 mM and 0.4 mM, respectively.

Kit components:

	100 reaction kit	500 reaction kit
HotStart-IT Probe qPCR Master Mix (2X)	2 x 1.25 ml	1 x 12.5 ml
25 mM MgCl ₂	1 x 1 ml	5 x 1 ml
ROX™ Passive Reference Dye	1 x 100 µl	1 x 500 µl
M-MLV RT	1 x 40 µl	1 x 200 µl
RNase Inhibitor (10 units/µl)	1 x 40 µl	1 x 200 µl
RNase-Free Water, DEPC-Treated	3 x 1 ml	1 x 15 ml
Brief protocol		

Shipping and storage:

Shipped on dry ice. Store at -20°C. Mix all components well prior to use. Light sensitive components should be protected from excessive light exposure.

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

Affymetrix, USB and HotStart-IT are registered trademarks of Affymetrix, Inc. Tested User Friendly is a trademark of Affymetrix, Inc. HotStart-IT Taq DNA Polymerase—Methods for using this product may be covered by US Patent No. 7,700,281. 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. TaqMan is a registered trademark of Roche Molecular Systems, Inc. ROX is a trademark of Applied Biosystems or its subsidiaries in the US and certain other countries. BHQ-1 is a registered trademark of Biosearch Technologies.

HotStart-IT Probe One-Step qRT-PCR Master Mix Kit

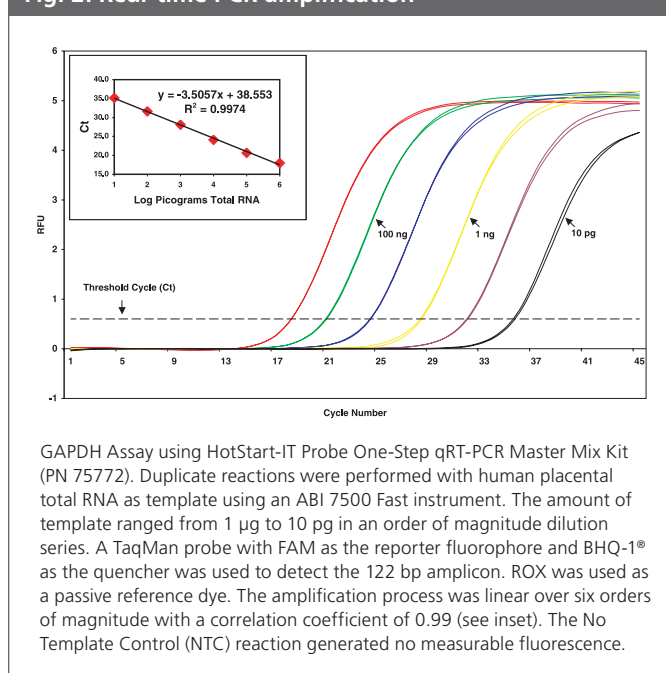
Product code	Pack size
75772	100 reactions
	500 reactions

One reaction is based on 50 µl PCR volume.

References:

- Goblet, C., Prost, E., and Whalen, R. G. (1989) *Nucleic Acids Res.* **17**, 2144.
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- Roth, M. J., Tanese, N., and Goff, S. P. (1985) *J. Biol. Chem.* **260**, 9326-9335.
- Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988) *Science* **239**, 487-491.
- Livak, K. J., Flood, S. J., Marmaro, J., Giusti, W., and Deetz, K. (1995) *PCR Methods Appl.* **4**, 357-362.
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Fig. 2. Real-time PCR amplification



GAPDH Assay using HotStart-IT Probe One-Step qRT-PCR Master Mix Kit (PN 75772). Duplicate reactions were performed with human placental total RNA as template using an ABI 7500 Fast instrument. The amount of template ranged from 1 µg to 10 pg in an order of magnitude dilution series. 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 six orders of magnitude with a correlation coefficient of 0.99 (see inset). The No Template Control (NTC) reaction generated no measurable fluorescence.

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