

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



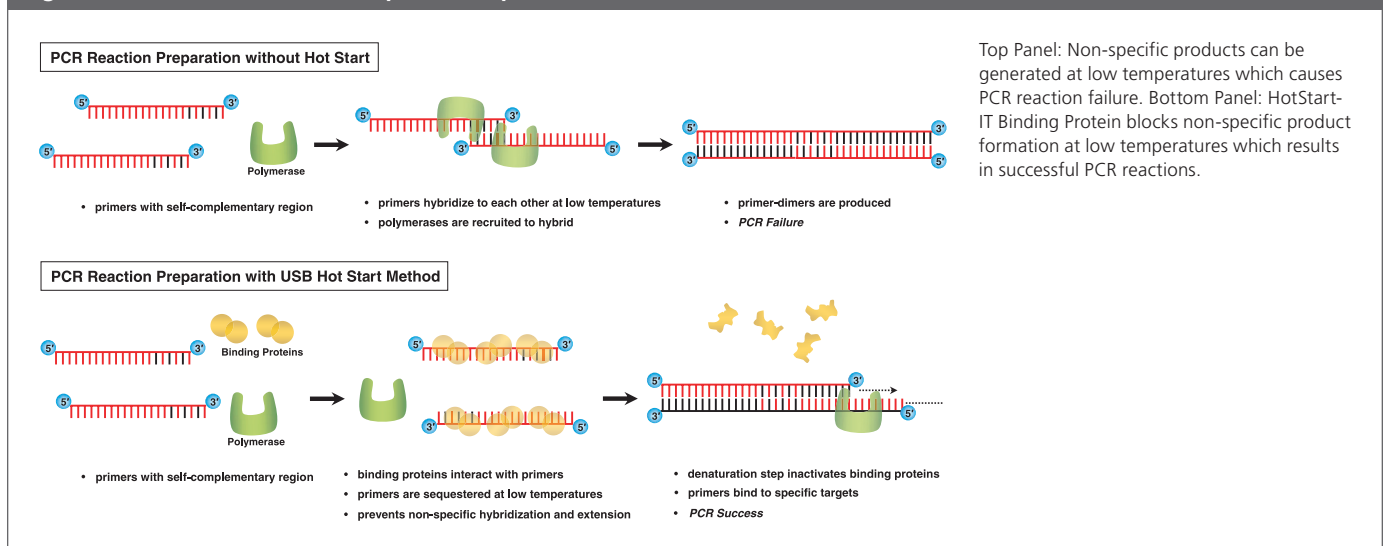
- **Based on innovative HotStart-IT technology**
 - Proprietary primer sequestration method
 - Increases specificity and prevents primer-dimer formation
 - No DNA template damage – no extensive heating step needed to denature hot start component
- **Convenient**
 - One-step, sequential reaction format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

USB HotStart-IT SYBR Green 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 SYBR Green 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, Ultrapure nucleotides, and SYBR Green I in an optimized reaction buffer. HotStart-IT SYBR Green 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 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). SYBR Green I Dye is used in this kit to detect any double-stranded DNA that accumulates during the amplification process and also allows melt-curve analyses. No fluorescent probes are required. Individual kit components have been carefully formulated to obtain optimal activity of M-MLV RT, Taq DNA Polymerase, and SYBR Green I to allow highly sensitive and specific detection of RNA transcripts from either total RNA or poly(A)+ mRNA. Separate tubes of the passive reference dyes, ROX™ and fluorescein, are included for added convenience to allow normalization of well-to-well variations.

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



Tested User Friendly™ functional test:

The HotStart-IT SYBR Green 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 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 SYBR Green qPCR Master Mix (2X), PN 75762

This Master Mix is a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, MgCl₂, Ultrapure nucleotides, and SYBR Green I in an optimized reaction buffer for use in real-time, quantitative PCR reactions. Magnesium and nucleotide concentrations are at 5 mM and 0.4 mM, respectively.

Kit components:

	100 reaction kit	500 reaction kit
HotStart-IT SYBR Green 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
Fluorescein 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.

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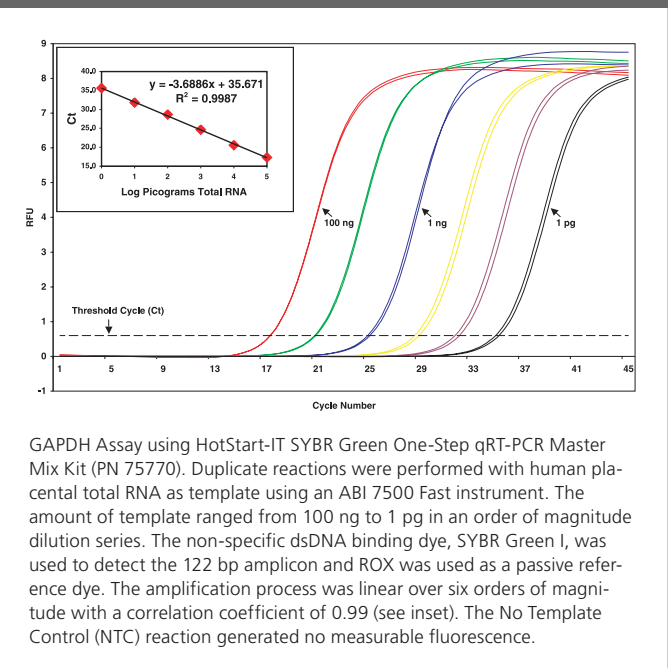
Product code	Pack size
75770	100 reactions
	500 reactions

One reaction is based on 50 µl PCR volume.

References:

- Goblet, C., Prost, E., and Whalen, R. G. (1989) *Nucleic Acids Res.* **17**, 2144.
- Sambrook, J. and Russell, D. W. (2001) "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, 8.46-8.53.
- Sellner, L. N., Coelen, R. J., and MacKenzie, J. S. (1992) *Nucleic Acids Res.* **20**, 1487-1490.
- 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.

Fig. 2. Real-time PCR amplification



GAPDH Assay using HotStart-IT SYBR Green One-Step qRT-PCR Master Mix Kit (PN 75770). Duplicate reactions were performed with human placental total RNA as template using an ABI 7500 Fast instrument. The amount of template ranged from 100 ng to 1 pg in an order of magnitude dilution series. The non-specific dsDNA binding dye, SYBR Green I, was used to detect the 122 bp amplicon and 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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