

USB® HotStart-IT® SYBR® Green qPCR Master Mix with UDG (2X)



- **Complete master mix with SYBR Green**
 - Compatible with standard SYBR filters
 - Multiple platform capability - includes separate tubes of ROX™ and Fluorescein Passive Reference Dyes to allow for normalization on different instruments
- **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
- **Carry-over contamination prevention with heat-labile UDG directly in master mix**
 - Optimized dUTP to dTTP ratio for enhanced sensitivity
- **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 SYBR Green qPCR Master Mix with Uracil-DNA Glycosylase (UDG) uses a novel hot start method designed and developed at Affymetrix called primer sequestration. With this method, a protein binds and sequesters 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 SYBR Green qPCR Master Mix with UDG is supplied as a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, MgCl₂, Ultra-pure nucleotides with an optimized dUTP to dTTP ratio, heat-labile UDG, and SYBR Green I for use in real-time quantitative PCR reactions (qPCR). Simply add DNA template, primers, and water and the reactions are ready for cycling. Separate tubes of passive reference dyes, ROX (for ABI and Stratagene instruments) and fluorescein (for BioRad instruments), are included for added convenience.

Since the mix contains dUTP and UDG, carryover contamination prevention can be performed, which is especially important for high-throughput applications⁽¹⁾. A heat-labile version of UDG that is irreversibly heat-inactivated is used instead of *E. coli* UDG, which has been shown to exhibit residual activity following PCR reactions⁽²⁾. The SYBR Green I dye detects any double-stranded DNA that accumulates during the amplification process.

HotStart-IT SYBR Green qPCR Master Mix with UDG 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.

Convenient

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

Carry-Over Contamination Prevention

Eliminate at least 10⁵ copies of dUTP-containing contaminating templates. The dUTP in the mix ensures that products which contain uracil are destroyed prior to subsequent amplification reactions by the enzymatic activity of the Uracil-DNA Glycosylase. After the initial denaturation step, the UDG is inactivated, and only the desired target sequences without dUTP are amplified.

Advantage with heat-labile UDG

The UDG used in the USB master mix is completely and irreversibly heat-inactivated due to the high-temperature cycling conditions. This maintains the integrity of the PCR products following reactions which is important if they are to be used in subsequent analyses such as gel electrophoresis, cloning, and/or sequencing.

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

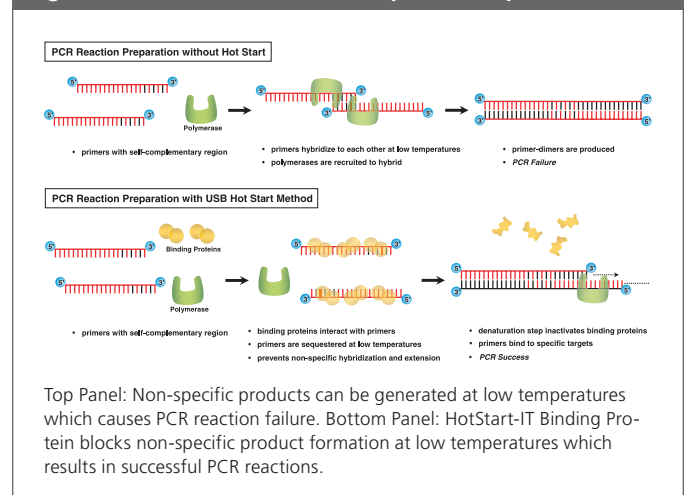
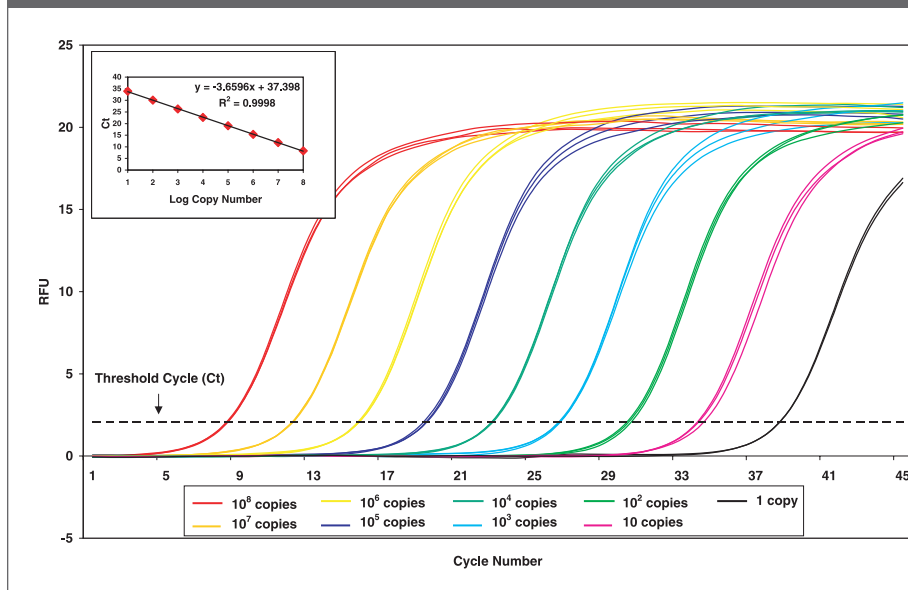


Fig. 2. Real-time PCR amplification



GAPDH Assay using HotStart-IT SYBR Green qPCR Master Mix with dUTP and UDG (PN 75760). Triplicate reactions were performed with a cloned region of the human GAPDH gene as template using an ABI 7500 Fast instrument. 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 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.

Higher specificity, sensitivity and broad dynamic range

The hot start feature minimizes amplification of non-specific products and primer-dimers. The SYBR Green I concentration has been carefully optimized for maximum sensitivity and can be used in melt-curve analyses. 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. 2).

Stable

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

Components:

HotStart-IT SYBR Green qPCR Master Mix with UDG (2X): 100 reactions (2 x 1.25 ml), 500 reactions (12.5 ml), 25 mM MgCl₂, ROX Passive Reference Dye, Fluorescein Passive Reference Dye

HotStart-IT SYBR Green qPCR Master Mix with UDG formulation (2X):

The mix combines USB HotStart-IT Taq DNA Polymerase, heat-labile UDG, SYBR Green I, MgCl₂, and Ultrapure nucleotides with an optimized dUTP to dTTP ratio in a unique buffer formulation. Magnesium and nucleotide concentrations are 5 mM and 0.4 mM each, respectively.

HotStart-IT SYBR Green qPCR Master Mix with UDG

Product code	Pack size
75760	100 reactions
	500 reactions

References:

- Longo, M. C., Berninger, M. S., and Hartley, J. L. (1990) *Gene* **93**, 125-128.
- Thornton, C. G., Hartley, J. L., and Rashtchian, A. (1992) *BioTechniques* **13**, 180-184.

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