



HotStart-IT.™  
Less Heat.  
No DNA Damage.  
We couldn't be  
more specific.

Real-Time PCR from USB

 **usb**®  
*Fueling Innovation  
in Life Science™*

## qPCR Carry Over Prevention

### HotStart-IT™ qPCR Master Mixes with UDG

#### Benefits

- **Effective Carry Over Contamination Prevention** –

Eliminate at least 10<sup>5</sup>

copies of dUTP-containing contaminating templates

- **Heat-labile UDG is included in master mixes**
  - UDG is completely and irreversibly heat-inactivated, unlike *E. coli* UDG
  - Optimized dUTP to dTTP ratio for enhanced sensitivity
- **All-in-one universal master mixes** – May be used on multiple instruments
- **Flexible** – Choose either SYBR®-based or probe-based master mixes

#### How It Works

HotStart-IT™ qPCR Master Mixes with UDG are supplied as 2X pre-mixed formulations containing HotStart-IT™ Taq DNA Polymerase, MgCl<sub>2</sub>, Ultrapure nucleotides with an optimized dUTP to dTTP ratio, and heat-labile UDG for use in real-time quantitative PCR reactions (qPCR) with either fluorescent probes or SYBR® Green Dye. Since the mix contains dUTP and UDG, carry-over contamination prevention can be performed, which is especially important for high-throughput applications.<sup>(1)</sup> 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 (UDG). 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.<sup>(2)</sup> After the initial denaturation step, the UDG is inactivated, and only the desired target sequences without dUTP are amplified. 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) and/or Fluorescein (for BioRad instruments) is included for added convenience.

#### References

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

### Advantages with HotStart-IT™ qPCR Master Mixes for Carry Over Prevention

- Includes novel USB primer-sequestration method, HotStart-IT™, to reliably prevent mispriming and primer-dimer formation
- Novel heat-labile UDG is completely and irreversibly heat-inactivated, unlike *E. coli* UDG
- Flexible use with a variety of instruments

#### Probe-Based Carry Over Prevention

HotStart-IT™ Probe 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. A separate tube of ROX™ Passive Reference Dye allows normalization of well-to-well variations that may occur independent of the reactions (*e.g.*, pipetting errors, detection system limitations, etc.).

#### HotStart-IT™ Probe qPCR Master Mix with UDG (2X)



- 50 µl reactions
- Includes 25mM MgCl<sub>2</sub> and ROX™ Passive Reference Dye

75764	100 reactions	\$155
	500 reactions	\$659

#### SYBR®-Based Carry Over Prevention

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 any instrument's standard SYBR® filter set (typically, FAM setting). The separate ROX™ and Fluorescein Passive Reference Dyes allow normalization of well-to-well variations that may occur independent of the reactions (*e.g.*, pipetting errors, detection system limitations, etc.).

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



- 50 µl reactions
- Includes 25mM MgCl<sub>2</sub> and ROX™ & Fluorescein Passive Reference Dyes

75760	100 reactions	\$165
	500 reactions	\$701

## HotStart-IT™ Technology

### HotStart-IT™

#### Hot start PCR amplification from USB

USB's novel hot start PCR method requires no extensive heat denaturation step. The result is no

damage to precious samples with increased specificity and yield. HotStart-IT™: highly sensitive to all your critical PCR applications.

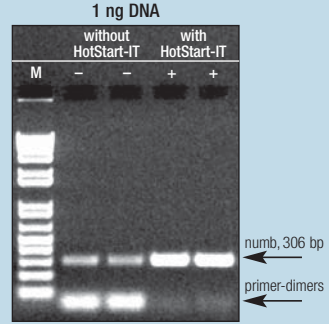
### Benefits

- **No DNA Template Damage** – No extensive heating step needed to denature hot start component
- **High Specificity** – Avoids non-specific products and primer-dimer formation
- **Convenient** – Room temperature reaction set-up

### How It Works

HotStart-IT™ Taq DNA Polymerase uses a novel hot start method designed and developed at USB 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, USB uses 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 mispriming 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.

### Increased specificity of HotStart-IT™ Taq DNA Polymerase



Results clearly demonstrate a shift from predominantly primer-dimers to the specific target when HotStart-IT™ is included in the reactions.

### HotStart-IT™ Taq DNA Polymerase



- Includes 10X PCR Reaction Buffer and 25mM MgCl<sub>2</sub>

71195	50 units	\$38
	250 units	\$145
	1,000 units	\$490
	5 x 250 units	\$638
	5,000 units	\$2,250

### HotStart-IT™ Taq Master Mix (2X)

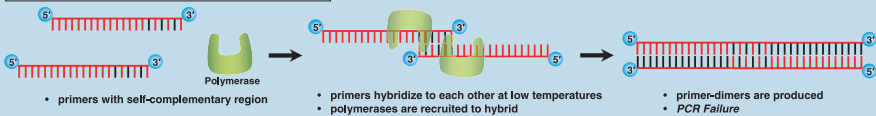


- 50 µl reactions

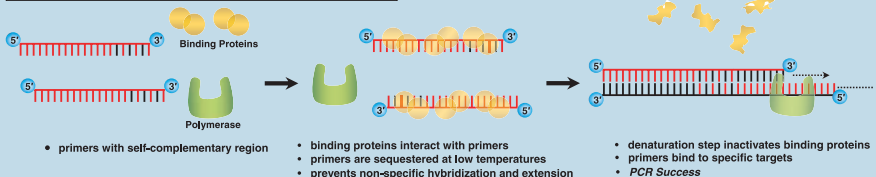
71196	25 reactions	\$34
	100 reactions	\$115
	500 reactions	\$525

### USB HotStart-IT™ Method: Primer Sequestration

#### PCR Reaction Preparation without Hot Start



#### PCR Reaction Preparation with USB Hot Start Method



## SYBR®-Based qPCR

### HotStart-IT™ SYBR® Green qPCR Master Mix

#### Benefits

- All-in-one universal 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
- 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  $\geq 0.95$

#### How It Works

HotStart-IT™ SYBR® Green qPCR Master Mix is supplied as a 2X pre-mixed formulation containing HotStart-IT™ Taq DNA Polymerase, MgCl<sub>2</sub>, Ultrapure nucleotides, and SYBR® Green I for use in real-time

quantitative PCR reactions (qPCR). The SYBR® Green I dye detects any double-stranded DNA that accumulates during the amplification process. The hot start feature enhances SYBR®-based qPCR reactions by reducing primer-dimer formation which increases specificity and sensitivity. Simply add DNA template, primers, and water and the reactions are ready for cycling. Separate tubes of the passive reference dyes, ROX (for ABI and Stratagene instruments) and fluorescein (for BioRad instruments), are included for added convenience.



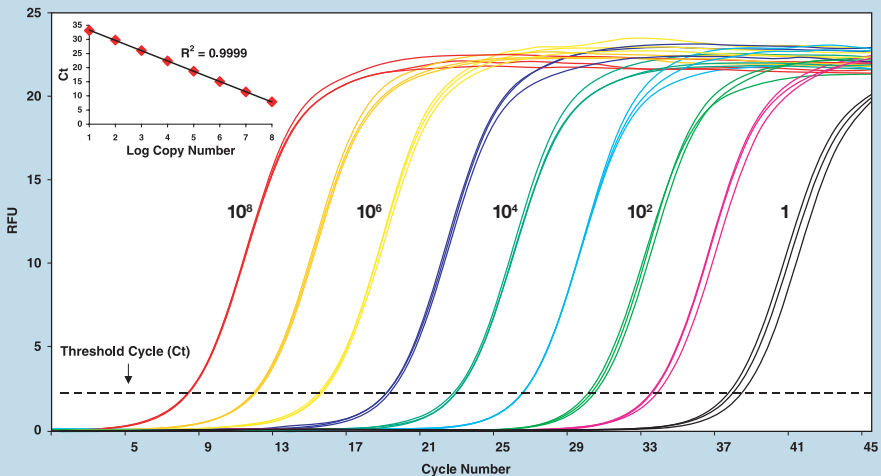
#### HotStart-IT™ SYBR® Green qPCR Master Mix (2X)



- 50 µl reactions
- Includes 25mM MgCl<sub>2</sub> and ROX™ & Fluorescein Passive Reference Dyes

75762	100 reactions	\$150
	500 reactions	\$638

#### Real-time PCR from 10<sup>8</sup> copies down to a single copy target using HotStart-IT™ SYBR® Green qPCR Master Mix



GAPDH Assay using HotStart-IT™ SYBR® Green qPCR Master Mix (PN 75762). 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.

## Probe-Based qPCR

### HotStart-IT™ Probe qPCR Master Mix

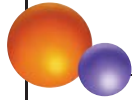
#### Benefits

- **All-in-one universal master mix for use with fluorescent probes**
  - Compatible with TaqMan® probes, Molecular Beacons, FRET probe pairs, etc.
  - Multiple platform capability – includes ROX™ Passive Reference Dye to allow normalization of well-to-well variations
- **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  $\geq 0.95$

#### How It Works

HotStart-IT™ Probe qPCR Master Mix is supplied as a 2X premixed formulation containing HotStart-IT™ Taq DNA Polymerase, MgCl<sub>2</sub>, and Ultrapure nucleotides for use in real-time quantitative PCR reactions (qPCR) with fluorescent probes. 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. 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.



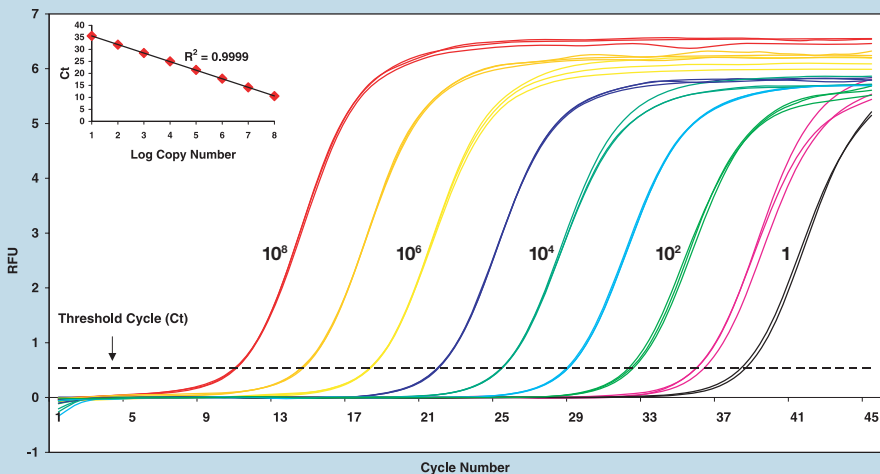
#### HotStart-IT™ Probe qPCR Master Mix (2X)



- 50 µl reactions
- Includes 25mM MgCl<sub>2</sub> and ROX™ Passive Reference Dye

75766	100 reactions	\$140
	500 reactions	\$595

#### Real-time PCR from 10<sup>8</sup> copies down to a single copy target using HotStart-IT™ Probe qPCR Master Mix



**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.

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Innovation in  
Life Science™

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Enzymes

Restriction  
Enzymes

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