USB® HotStart-IT® SYBR® Green qPCR Master Mix (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 hotstart 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 SYBR Green qPCR Master Mix 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 is supplied as a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, MgCl₂, Ultrapure nucleotides, 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 the passive reference dyes, ROX (for ABI and Stratagene instruments) and Fluorescein, (for BioRad instruments) are included for added convenience.

The master mix has SYBR Green I dye which 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. HotStart-IT SYBR Green 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 HotStart-IT SYBR Green PCR Master Mix with UDG (2X), PN 75760.

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

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.

---

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

Top Panel: Non-specific products can be generated at low temperatures which causes PCR reaction failure. Bottom Panel: HotStart-IT Binding Protein blocks non-specific product formation at low temperatures which results in successful PCR reactions.
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.

Functional test:
Real-time PCR reactions were performed on an ABI 7500 Fast Instrument using primers 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 to 10⁷ template copies) must be greater than or equal to 0.95.

HotStart-IT SYBR Green qPCR Master Mix (2X):
The mix combines USB HotStart-IT Taq DNA Polymerase, SYBR Green I, MgCl₂, and Ultrapure nucleotides in a unique buffer formulation. Magnesium and nucleotide concentrations are 5 mM and 0.4 mM each, respectively.

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

Brief protocol
Shipping and storage:
Shipped on dry ice. Store at -20°C. Mix well prior to use.

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

<table>
<thead>
<tr>
<th>Product code</th>
<th>Pack size</th>
</tr>
</thead>
<tbody>
<tr>
<td>75762</td>
<td>100 reactions</td>
</tr>
<tr>
<td></td>
<td>500 reactions</td>
</tr>
</tbody>
</table>