

HotStart-IT® master mix series

High dynamic range and high sensitivity qPCR and qRT-PCR

High dynamic range and high sensitivity by primer sequestration

HotStart-IT® master mix series use a novel hot start method developed at USB called primer sequestration (Fig. 1). This novel hot start feature increases the specificity and sensitivity of SYBR-based qRT-PCR reactions by substantially reducing primer-dimer formation. With this method, the HotStart-IT protein binds and sequesters primers at lower temperatures making them unavailable for use by Taq DNA Polymerase. Following the first heat denaturation step, the primer binding protein is inactivated and the primers are released.

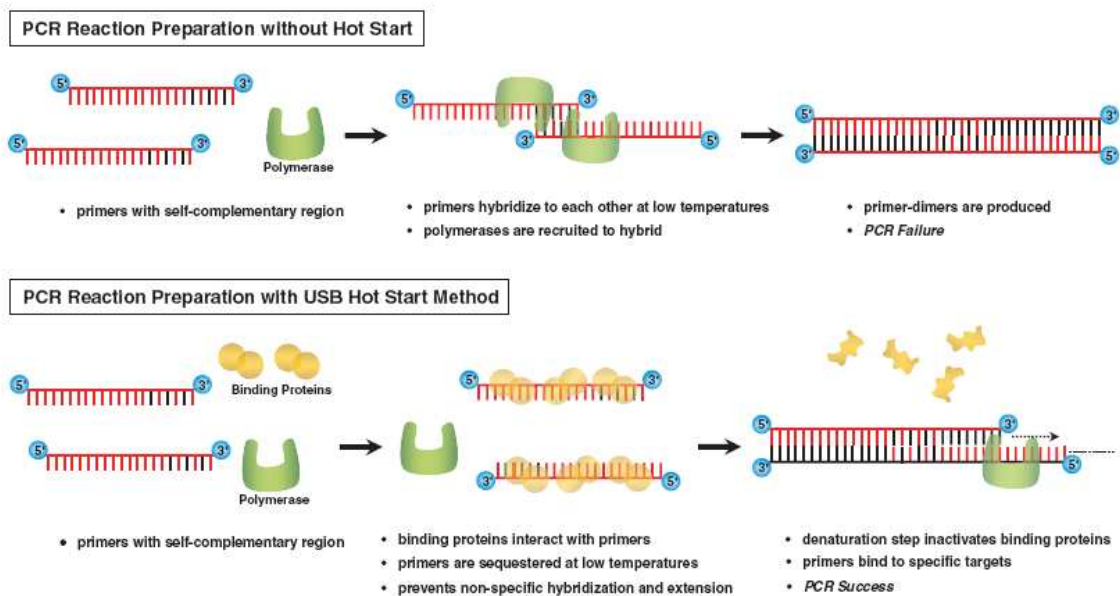


Fig. 1. HotStart-IT® method. Top Panel: Non-specific products can be generated at low temperatures which causes PCR reaction failure. Bottom Panel: HotStart-IT® binding protein blocks non-specific product formation at low temperatures which results in successful PCR reactions.

Since side products are greatly avoided by primer sequestration, low background noise allows high sensitivity for fewer than 10 target copies, and therefore large linear dynamic range of 6 to 7 of magnitude (Fig. 2).

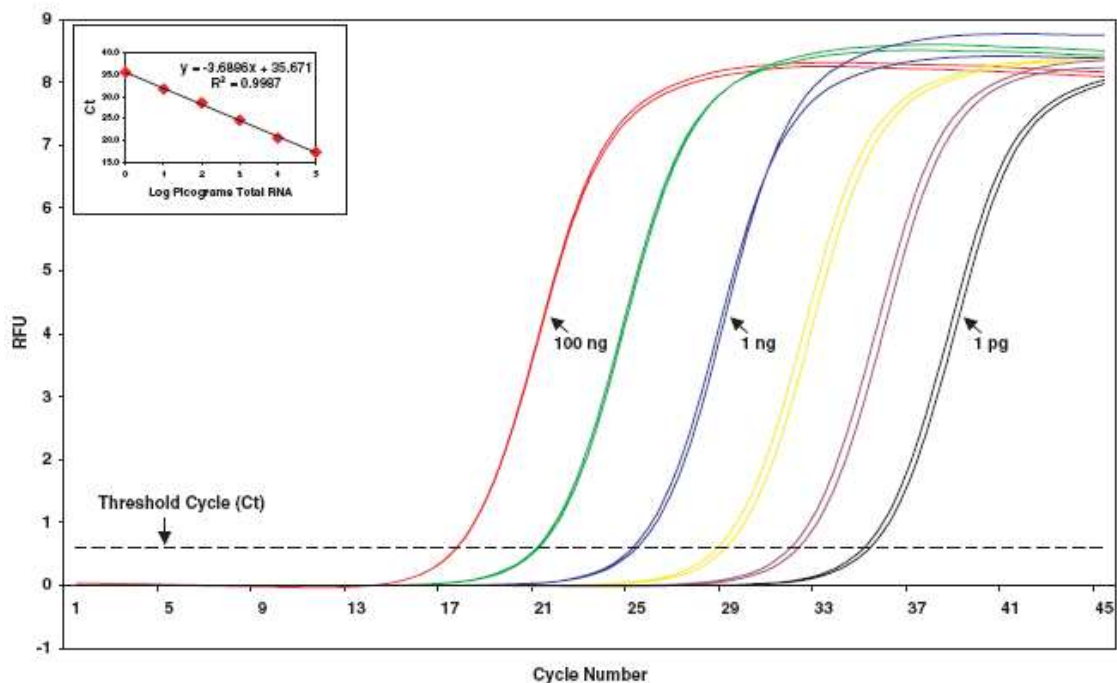


Fig. 2. Real-time PCR Amplification using HotStart-IT® SYBR® Green One-Step qRT-PCR Master Mix Kit (PN 75770). GAPDH Assay using HotStart-IT® SYBR® Green One-Step qRT-PCR Master Mix Kit (PN 75770). Duplicate reactions were performed with human placental total RNA as template using an ABI 7500 Fast instrument. 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.

Complete and convenient

The master mix contains HotStart-IT® Taq polymerase and buffer solution that are optimum for qPCR and qRT-PCR. Passive reference dyes (ROX™ and fluorescein) are also provided in HotStart-IT® series. SYBR® Green is additionally included in the master mix for SYBR® Green specific reactions. On the other hand, extra reverse transcriptase and RNase inhibitors are provided for qRT-PCR.

You just simply add the primers and template to the master mix. You can also add other components for your applications. A brief protocol is already available in the kit for your reference.

Products of HotStart-IT® series:

- 1) [HotStart-IT® SYBR® Green qPCR Master Mix \(2X\) \(Cat. No.: 75762\)](#)
- 2) [HotStart-IT® SYBR® Green qPCR Master Mix with UDG \(2X\) \(Cat. No.: 75760\)](#)
- 3) [HotStart-IT® Probe qPCR Master Mix \(2X\) \(Cat. No.: 75766\)](#)
- 4) [HotStart-IT® Probe qPCR Master Mix with UDG \(2X\) \(Cat. No.: 75764\)](#)
- 5) [HotStart-IT® SYBR® Green One-Step qRT-PCR Master Mix Kit \(Cat. No.: 75770\)](#)
- 6) [HotStart-IT® Probe One-Step qRT-PCR Master Mix Kit \(Cat. No.: 75772\)](#)