Affymetrix® | USB® Products

PCR Tools

USB® HotStart-IT® for real-time PCR

Reliable performance

- Functionally tested to deliver a linear dynamic range of seven orders of magnitude
- USB HotStart-IT Taq DNA Polymerase only needs a 2 minute heat activation step
- Detect single copy targets
- Matches performance of popular master mixes such as ABI TaqMan® Universal PCR Master Mix

Value and convenience

- Price per reaction is 25% less than TaqMan Universal PCR Master Mix
- Multiple formulations available for different detection chemistries

USB HotStart-IT master mixes for real-time PCR enable robust quantitative detection of nucleic acids. Designed to provide consistent results, HotStart-IT qPCR master mixes perform equally well as traditional real-time PCR reagents from other suppliers (Fig. 1) at a better price per reaction. And, unlike chemically modified Taq DNA Polymerase, USB HotStart-IT Taq DNA Polymerase only needs a 2 minute heat activation step. HotStart-IT qPCR master mixes are functionally tested to ensure linear performance over a broad dynamic range of seven orders of magnitude (Fig. 2).

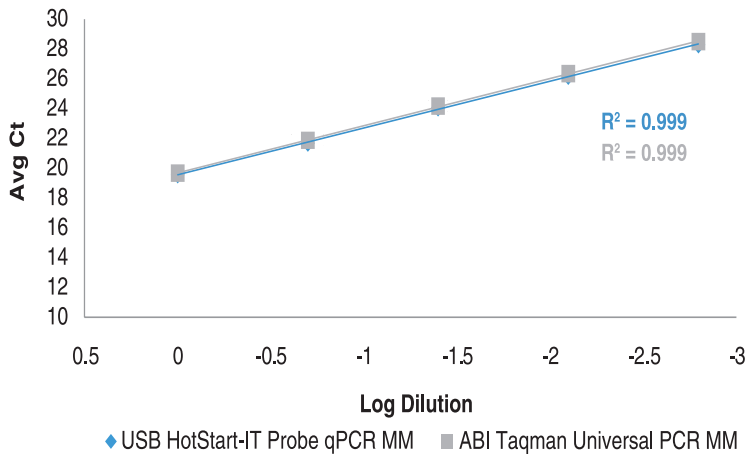
USB HotStart-IT qPCR reagents utilize a novel primer binding protein to achieve a hot start. Primers are sequestered at lower temperatures which prevent the formation of non-specific products and primer dimers. The HotStart-IT binding protein enables efficient and specific amplification of the desired product, resulting in more sensitive real-time PCR. In addition, HotStart-IT master mixes only need a 2 minute heat activation step in contrast to chemically modified polymerases that require excessive heat activation steps over 10 minutes.

Formulations are available for either specific (*e.g.* TaqMan) or non-specific (*e.g.* SYBR®) detection chemistries. Mixes containing Uracil-DNA Glycosylase are also available if carry-over contamination prevention is desired. Additionally, HotStart-IT qPCR reagents are available with the passive reference dye, ROX™, already mixed in the master mix, or with both ROX and fluorescein included in separate tubes for maximum flexibility.

For expression analysis of RNA using qRT-PCR, HotStart-IT qRT-PCR master mixes are available in convenient one-step formats to perform both cDNA synthesis and real-time PCR in the same tube. If a two-step qRT-PCR workflow is desired, the USB First Strand cDNA Synthesis Kit for Real-Time PCR can be used to reliably generate cDNA prior to qPCR. This kit eliminates end bias that can be introduced by traditional reverse transcription methods utilizing either oligo(dT) or random hexamers.

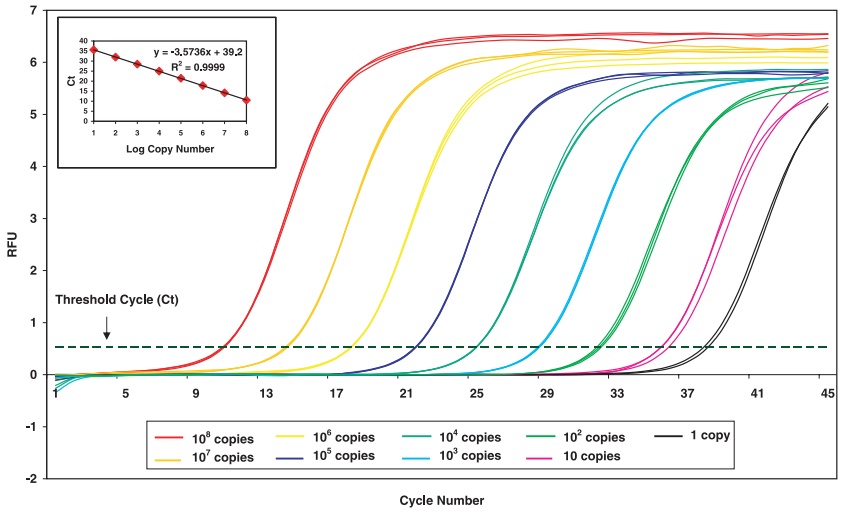
Try USB HotStart-IT master mixes and maximize the value of your real-time PCR.

Fig. 1. Performance comparison of HotStart-IT Probe qPCR Master Mix (PN 75766)



Comparison of USB HotStart-IT Probe qPCR master mix vs. ABI TaqMan Universal PCR master mix. TaqMan assay under standard ABI cycling conditions to quantify expression of reference gene. All reactions were performed in duplicate. Data courtesy of Anne Jedlicka, Johns Hopkins Malaria Research Institute, Baltimore, MD.

Fig. 2. Real-time PCR Amplification using HotStart-IT Probe qPCR Master Mix Kit (PN 75766)



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. The No Template Control (NTC) reaction generated no measurable fluorescence.

HotStart-IT real-time PCR master mixes

	100 x 50 µl reactions	500 x 50 µl reactions
HotStart-IT Probe qPCR Master Mix (2X), PN 75766		
HotStart-IT SYBR Green qPCR Master Mix (2X), PN 75762		
HotStart-IT Probe qPCR Master Mix with UDG (2X), PN 75764		
HotStart-IT SYBR Green qPCR Master Mix with UDG (2X), PN 75760		

HotStart-IT one-step qRT-PCR master mixes

	100 x 50 µl reactions	500 x 50 µl reactions
HotStart-IT Probe One-Step qRT-PCR Master Mix Kit, PN 75772		
HotStart-IT SYBR Green One-Step qRT-PCR Master Mix Kit, PN 75770		

Reverse Transcription - cDNA Synthesis

	50 reactions
First Strand cDNA Synthesis Kit for Real-Time PCR, PN 75780	

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