

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

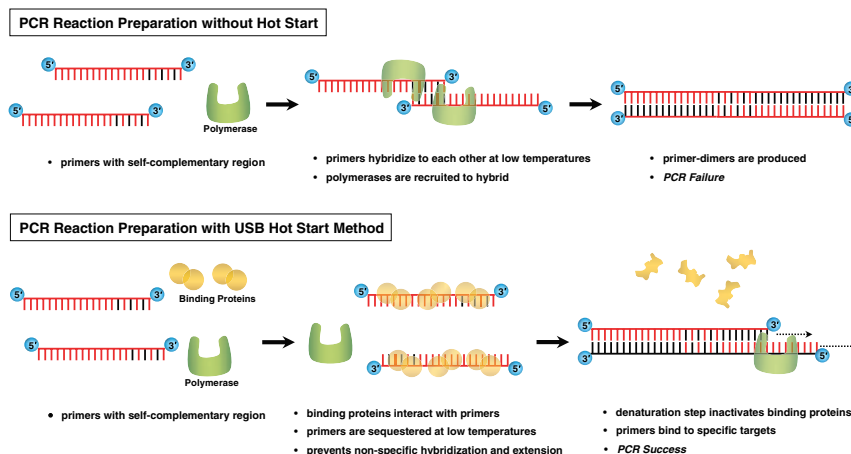


- **All-in-one universal master mix for use with fluorescent probes**
 - Compatible with TaqMan® probes, Molecular Beacons, FRET probe pairs, etc.
 - Multiple platform capability - includes ROX™ Reference Dye to allow normalization of well-to-well variations
- **Carry-Over Contamination Prevention with heat-labile UDG directly in master mix**
 - UDG is completely and irreversibly heat-inactivated unlike *E.coli* UDG
 - 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 correlation coefficient = 0.95
- **Based on USB novel Hot Start method of primer sequestration**
 - No DNA template damage - no extensive heating step needed to denature hotstart component
 - Avoids non-specific products & primer-dimer formation
 - Room temperature reaction set-up

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

HotStart-IT™ Probe qPCR Master Mix with Uracil-DNA Glycosylase (UDG) uses a novel hot start method designed and developed at USB 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 Probe qPCR Master Mix with UDG is supplied as a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, MgCl₂, Ultrapure nucleotides with an optimized dUTP to dTTP ratio, and heat-labile UDG for use in real-time quantitative PCR reactions (qPCR) with fluorescent 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.

Fig. 1. USB HotStart-IT Method: Primer Sequestration



Since the mix contains dUTP and UDG, carry-over 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 mix is formulated for use with fluorescent probes such as TaqMan® Probes, Molecular Beacons, and others⁽³⁻⁴⁾. Since fluorescent probes are designed to hybridize to the target of interest, detection specificity is greatly increased relative to nonspecific 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. 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.

Convenient

Save time and reduce potential contamination errors by eliminating several pipetting steps. For a 50 µl reaction, simply add 25 µl of master mix to primers and probe(s), 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.

Multiple Platform Compatibility

Specialized buffer with optimal MgCl₂ concentration and dUTP to dTTP ratio performs well on a variety of platforms. Also, the 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.).

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 also included in the mix. 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, unlike *E. coli* UDG used in most other products, 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.

Higher Specificity, Sensitivity and Broad Dynamic Range

The hot start feature minimizes amplification of non-specific products and primer-dimers. The reaction buffer with optimum MgCl₂ concentration is specially designed for robust probe hybridization and efficient cleavage of TaqMan[®] probes. 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).

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.

Stable

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

Components

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

100 reactions 2 x 1.25 ml
500 reactions 12.5 ml

25mM MgCl₂

ROX Passive Reference Dye

Tested User Friendly™ Functional Tests:

Real-time PCR reactions were performed on an ABI 7500 Fast Instrument using primers and TaqMan[®] probe 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.

Additionally, carry-over contamination tests of the UDG activity in the mix were performed. Product specifications require removal of greater than or equal to 10⁵ dUTP-containing template copies per reaction.

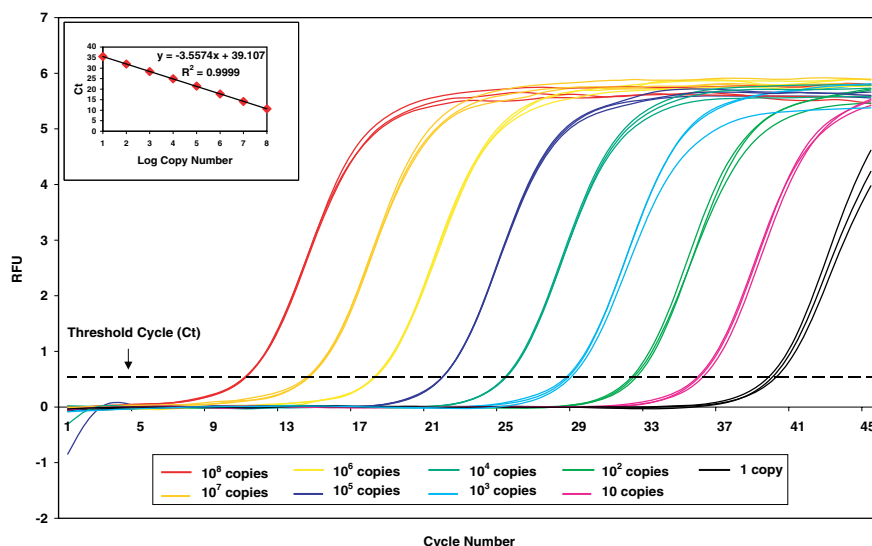
The mix combines USB HotStart-IT Taq DNA Polymerase (with 5'→3' exonuclease activity), heat-labile UDG, MgCl₂, and Ultrapure nucleotides with an optimized dUTP to dTTP ratio in a unique buffer formulation. Magnesium and nucleotide concentrations are 6mM and 0.4mM each, respectively.

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.
3. Livak, K. J., Flood, S. J., Marmaro, J., Giusti, W., and Deetz, K. (1995) *PCR Methods Appl.* **4**, 357-362.
4. Tyagi, S., and Kramer, F. R. (1996) *Nat. Biotechnol.* **14**, 303-308.

Product Code	Pack Size	List Price
75764	100 reactions	\$155.00
	500 reactions	\$659.00

Fig. 2. Real-time PCR Amplification using HotStart-IT™ Probe qPCR Master Mix with UDG (PN 75764).



GAPDH Assay using HotStart-IT Probe qPCR Master Mix with dUTP and UDG (PN 75764). 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.

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Taq DNA Polymerase – Patent pending. Taq DNA Polymerase – sold under licensing arrangements with Applied Biosystems. Purchase is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler. The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and F. Hoffmann-La Roche Ltd. UDG – Purchase of this product is accompanied by a limited license under U.S. Patent Nos. 5,035,996; 5,683,896; 5,945,313; 6,518,026 and 6,287,823 and corresponding foreign patents. TaqMan is a registered trademark of Roche Molecular Systems, Inc. ROX is a trademark of Applied Biosystems Corporation or its subsidiaries in the U.S. and certain other countries.



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