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HotStart-IT® Probe qPCR Master Mix (2X)

Tested User Friendly™
Product Number 75766
Brief Protocol

HotStart-IT® Probe qPCR Master Mix is a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, $MgCl_2$, and Ultrapure nucleotides for use in real-time quantitative PCR reactions with fluorescent probes (*e.g.* TaqMan® probes, Molecular Beacons, etc.). A separate tube of the passive reference dye ROX™ is also included.

Protocol

This protocol applies to a single reaction where only template, primers, probe(s) and water need to be added to the master mix. For multiple reactions, increase the volumes of the reaction components proportionally.

1. Thaw the master mix and other frozen reagents at room temperature. Mix thoroughly, briefly spin to collect tube contents and then place on ice.
2. Assemble reaction tubes or plates on ice or at room temperature, whichever is more convenient.

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3. This table shows recommended component volumes:

Component	Volume for 50 µl reaction	Volume for 20 µl reaction	Final Concentration
HotStart-IT® Probe qPCR Master Mix (2X)	25 µl	10 µl	1X (3mM $MgCl_2$)
10µM Forward Primer	0.5-5.0 µl	0.2-2.0 µl	0.1-1.0µM*
10µM Reverse Primer	0.5-5.0 µl	0.2-2.0 µl	0.1-1.0µM*
10µM Fluorescent Probe(s)	0.5-2.5 µl	0.2-1.0 µl	0.1-0.5µM*
Template DNA	X µl	X µl	as needed, < 500 ng†
ROX™ Passive Reference Dye *(optional: for ABI and Stratagene instruments)*	see included Passive Reference Dye protocol for details		
Water, PCR-Qualified	up to 50 µl	up to 20 µl	NA

*Optimal primer concentration is 0.2µM. In order to avoid primer-dimers and non-specific products, use ≤ 0.5µM. Start with a probe concentration of 0.1µM, although some optimization is generally required to determine the proper concentration.

†If template is cDNA from a first-strand synthesis reaction that has not been purified or diluted, do not exceed 10% of the final reaction volume (*i.e.* 5 µl for a 50 µl reaction). Dilute cDNA at least 1:10 in water to obtain best results.

4. *Optional*—If optimizing Mg concentration, add 2.0 µl of 25mM $MgCl_2$ per 50 µl reaction for each additional 1mM Mg required. Subtract this volume from the amount of water needed.
5. Cap tubes or seal plates with optically clear caps or film. Mix tubes or plates by gentle vortexing and then spin to collect contents without bubbles (*e.g.* 2-5 min at 1,000-2,000 x *g*).

6. The following tables show recommended cycling conditions:

Standard Cycling Program for TaqMan® Probes

1 cycle of: 95°C for 2 min: HotStart Binding Protein inactivation.
35-45 cycles of: 95°C for 15 sec 60°C for 30-60 sec: Acquire real-time fluorescence data during this step.

Fast Cycling Program (*e.g.* ABI 7500 in Fast Mode)

1 cycle of: 95°C for 2 min: HotStart Binding Protein inactivation.
35-45 cycles of: 95°C for 3-5 sec 60°C for 15-30 sec: Acquire real-time fluorescence data during this step.

Cycling Program for FRET Probes or Molecular Beacons

1 cycle of: 95°C for 2 min: HotStart Binding Protein inactivation.
35-45 cycles of: 95°C for 15 sec 50-60°C for 15-30 sec: Annealing temperature should be about 5°C below the T_m of the probe(s). Acquire real-time fluorescence data during this step. 72°C for 15-60 sec

Note: Due to the inherent specificity of TaqMan® probes, melt-curve analysis is not performed. Some probes such as FRET and Molecular Beacons or LUX™ primers may use melt-curve analyses. Consult the thermal cycler manual and probe/primer manufacturer recommendations for details.

7. If desired, confirm that specific PCR products have been generated by agarose gel electrophoresis. Amplicons may be detected on gels with ethidium bromide.