

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

1 cycle of: 25°C for 10 min: UDG treatment for carry-over contamination prevention.
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.
Melt-Curve Analysis: Recommended to distinguish specific products from non-specific ones. Consult thermal cycler manual 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 or without ethidium bromide using SYBR® Green I included in the reaction mix.

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## HotStart-IT™ SYBR® Green qPCR Master Mix with UDG (2X)



### Tested User Friendly™ Product Number 75760 Brief Protocol

HotStart-IT™ SYBR® Green qPCR Master Mix with UDG is a 2X pre-mixed formulation containing HotStart-IT Taq DNA Polymerase, MgCl<sub>2</sub>, Ultrapure nucleotides with an optimized dUTP to dTTP ratio, heat-labile Uracil-DNA Glycosylase (UDG), and SYBR Green I for use in real-time quantitative PCR reactions. Separate tubes of the passive reference dyes ROX™ and fluorescein are also included. Since the mix contains dUTP and UDG, carryover contamination prevention can be performed.

#### Protocol

This protocol applies to a single reaction where only template, primers 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.

*(continued on next page)*



3. This table shows recommended component volumes:

Component	Volume for 50 $\mu$ l reaction	Volume for 20 $\mu$ l reaction	Final Concentration
HotStart-IT™ SYBR® Green qPCR Master Mix with UDG (2X)	25 $\mu$ l	10 $\mu$ l	1X (2.5mM MgCl <sub>2</sub> )
10 $\mu$ M Forward Primer	0.5-5.0 $\mu$ l	0.2-2.0 $\mu$ l	0.1-1.0 $\mu$ M*
10 $\mu$ M Reverse Primer	0.5-5.0 $\mu$ l	0.2-2.0 $\mu$ l	0.1-1.0 $\mu$ M*
Template DNA	X $\mu$ l	X $\mu$ l	as needed, < 500 ng <sup>†</sup>
Passive Reference Dye (optional: ROX for ABI and Stratagene; Fluorescein for Bio-Rad)	see included Passive Reference Dye protocols for details		
Water, PCR-Qualified	up to 50 $\mu$ l	up to 20 $\mu$ l	NA

\*Optimal primer concentration is 0.2 $\mu$ M. In order to avoid primer-dimers and non-specific products, use  $\leq$  0.5 $\mu$ M.

<sup>†</sup>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  $\mu$ l for a 50  $\mu$ l reaction). Dilute cDNA at least 1:10 in water to obtain best results.

4. *Optional*—If optimizing Mg concentration, add 2.0  $\mu$ l of 25mM MgCl<sub>2</sub> per 50  $\mu$ l 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

1 cycle of: 25°C for 10 min: UDG treatment for carry-over contamination prevention.
1 cycle of: 94-95°C for 2 min: HotStart Binding Protein inactivation.
35-45 cycles of: 94-95°C for 15 sec 55°C for 15-30 sec: Annealing temperature should be about 5°C below T <sub>m</sub> of primers. 72°C for 30-60 sec: Acquire real-time fluorescence data during this step.
<i>(Optional)</i> X°C for 15 sec: If primer-dimers are a problem, instead of collecting real-time data at the extension temperature, collect data at a temperature which is about 3-5°C below the T <sub>m</sub> of the amplicon but above the T <sub>m</sub> of the primer-dimers.
Melt-Curve Analysis: Recommended to distinguish specific products from non-specific ones. Consult thermal cycler manual for details.

#### Cycling Program for Annealing/ Extension Step Combined

1 cycle of: 25°C for 10 min: UDG treatment for carry-over contamination prevention.
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.
Melt-Curve Analysis: Recommended to distinguish specific products from non-specific ones. Consult thermal cycler manual for details.