Additional Products

	Amount	Catalog No.
Custom primers	Visit **www.lifetechnologies.com/oligos**	
SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR	50 rxns	11752-050
	250 rxns	11752-250
SYBR® GreenER™ qPCR SuperMix for iCycler®	100 rxns	11761-100
	1250 rxns	11761-500
SYBR® GreenER™ qPCR SuperMix Universal	250 rxns	11762-100
	1250 rxns	11762-500

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invitrogen
by life technologies

SYBR® GreenER™ qPCR SuperMix for ABI PRISM®

Catalog. nos.
11760-100
11760-500

Size
100 reactions
500 reactions

Store at 2°C to 8°C

Publication Part number 11760.pps MAN0001023 **Revision Date** 27 January 2012

Description

SYBR® GreenER™ qPCR SuperMix for ABI PRISM® is a ready-to-use cocktail containing all components, except primers and template, for real-time quantitative PCR (qPCR) on ABI real-time instruments that support normalization with ROX Reference Dye at a final concentration of 500 nM. It combines a chemically modified "hot-start" version of *Taq* DNA polymerase with integrated uracil DNA glycosylase (UDG) carryover prevention technology and a novel fluorescent dye to deliver excellent sensitivity in the quantification of target sequences, with a linear dose response over a wide range of target concentrations. Volumes are provided for 100 or 500 amplification reactions of 50 µL each.

SYBR® GreenER™ qPCR SuperMix for ABI PRISM® is supplied at a 2X concentration and contains hot-start *Taq* DNA polymerase, SYBR® GreenER™ fluorescent dye, 1 µM ROX Reference Dye, $MgCl_2$, dNTPs (with dUTP instead of dTTP), UDG, and stabilizers. The SuperMix formulation can quantify fewer than 10 copies of a target gene, has a broad dynamic range, and is compatible with melting curve analysis.

- The fluorescent double-stranded DNA (dsDNA) binding dye in the SuperMix provides both higher sensitivity and lower PCR inhibition than SYBR® Green I dye. It can be used on real-time PCR instruments calibrated for SYBR® Green I dye without any change of filters or settings. In qPCR, as dsDNA accumulates, the SYBR® GreenER™ qPCR SuperMix dye generates a fluorescent signal that is proportional to the DNA concentration (Wittwer, 1997; Ishiguro, 1995).
- The *Taq* DNA polymerase provided in the SuperMix has been chemically modified to block polymerase activity at ambient temperatures, allowing room-temperature setup and long-term storage at 4°C. Activity is restored after a 10-minute incubation in PCR cycling, providing an automatic hot start for increased sensitivity, specificity, and yield (Chou, 1992).
- UDG and dUTP in the SuperMix prevent the reamplification of carryover PCR products between reactions (Longo, 1990). dUTP ensures that any amplified DNA will contain uracil, while UDG removes uracil residues from single- or double-stranded DNA. A UDG incubation step before PCR cycling destroys any contaminating dU-containing product from previous reactions (Lindahl, 1977). UDG is then inactivated by the high temperatures during normal PCR cycling, thereby allowing the amplification of genuine target sequences.
- ROX is included at a final concentration of 500 nM to normalize the fluorescent signal on instruments that are compatible with this option. ROX can adjust for non-PCR-related fluctuations in fluorescence between reactions, and provides a stable baseline in multiplex reactions. It is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester.

Component	100-rxn Kit	500-rxn Kit
SYBR® GreenER™ qPCR SuperMix for ABI PRISM®	2 × 1.25 mL	12.5 mL

Shipping and Storage

The SuperMix is shipped on dry ice and should be stored at 2ºC to 8°C. Storage at –30ºC to –10°C may extend shelf life.

Handling Conditions

Minimize exposure of SYBR® GreenER® qPCR SuperMix for ABI PRISM® to direct light. Exposure to direct light for an extended period of time may result in loss of fluorescent signal intensity.

Two-Step qRT-PCR Kits

This SuperMix is also included with the SYBR® GreenER™ Two-Step qRT-PCR Kit for ABI PRISM®, catalog nos. 11763-100 and 11763-500.

Important Parameters

Instrument compatibility

This kit can be used with ABI real-time instruments that are compatible with ROX Reference Dye at a final concentration of 500 nM. These instruments include the ABI PRISM® 7000, 7700, and 7900HT; the ABI 7300 Real-Time PCR System; and the ABI GeneAmp® 5700.

Note: This kit is *not* compatible with instruments that use ROX at a final concentration lower than 500 nM, including the ABI 7500. For these instruments, we recommend SYBR® GreenER™ qPCR SuperMix Universal, which includes ROX as a separate tube that can be added at the required concentration (see **Additional Products**, page 4).

Intended Use: For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Important Parameters, continued

Template

cDNA

- For two-step qRT-PCR, use undiluted or diluted cDNA generated from up to 1 µg of total RNA. For cDNA synthesis, we recommend the SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR (see **Additional Products**, page 4).
- A maximum of 10% of the qPCR reaction volume may be undiluted cDNA (e.g., for a 50-µL qPCR, use up to 5 µL of undiluted cDNA).
- Note that detecting high-abundance genes in undiluted cDNA may result in very low CTs in qPCR, leading to reduced quantification accuracy. Prepare a dilution series of the cDNA template for the most accurate results.

Plasmid and genomic DNA

Use up to 100 ng of genomic DNA or 10–10^7 copies of plasmid DNA in a 10-µL volume. Note that 1 µg of plasmid DNA contains 9.1×10^{11} copies divided by the plasmid size in kilobases.

Primers

Primer design is one of the most important parameters when using SYBR® GreenER™ qPCR SuperMix. We strongly recommend using a primer design program such as OligoPerfect™, available on the Web at **www.lifetechnologies.com/oligos**, or Vector NTI™. When designing primers, the amplicon length should be approximately 80–250 bp. A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 100 and 500 nM.

DNA polymerase activation time

The hot-start DNA polymerase is activated in the 10-minute incubation at 95°C before PCR cycling.

Melting curve analysis

Melting curve analysis should always be performed following real-time qPCR to identify the presence of primer dimers and analyze the specificity of the reaction. Program your instrument for melting curve analysis using the instructions provided with your specific instrument.

Magnesium concentration

Magnesium chloride is included in the SuperMix at an optimized concentration for qPCR.

General Protocol for ABI Instruments

Follow the general protocol below for qPCR on compatible ABI real-time instruments (see **Instrument Compatibility**, page 1).

1. Program your real-time instrument as shown below. Optimal temperatures and incubation times may vary.

   50ºC for 2 minutes hold (UDG incubation)
   95ºC for 10 minutes hold (UDG inactivation and DNA polymerase activation)
   40 cycles of:
   95ºC, 15 seconds
   60ºC, 60 seconds

   Melting curve analysis: Refer to instrument documentation

2. For each reaction, add the following to a 0.2-mL microcentrifuge tube or each well of a PCR plate. A standard 50-µL reaction size is provided; component volumes can be scaled as desired (e.g., scaled down to a 20-µL reaction volume for 384-well plates).

   For multiple reactions, prepare a master mix of common components, add the appropriate volume to each tube or plate well, and then add the unique reaction components (e.g., template). **Note:** Preparing a master mix is strongly recommended in qRT-PCR to reduce pipetting errors.

Component	Single rxn	Notes
SYBR® GreenER™ qPCR SuperMix for ABI PRISM®	25 µL	1X final conc.
Forward primer, 10 µM	1 µL	200 nM final conc.
Reverse primer, 10 µM	1 µL	200 nM final conc.
Template (up to 100 ng of genomic DNA, 10–10^7 copies of plasmid DNA, or cDNA generated from up to 1 µg of total RNA)	5–10 µL	max. 10% v/v undiluted cDNA
DEPC-treated water	to 50 µL	

3. Cap or seal the reaction tube/PCR plate, and gently mix. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.
4. Place reactions in a preheated real-time instrument programmed as described above. Collect data and analyze results.

Troubleshooting

Problem	Possible Cause	Solution
Signals are present in no-template controls, and/or multiple peaks are present in the melting curve graph	Template or reagents are contaminated by nucleic acids (DNA, cDNA)	Use melting curve analysis and/or run the PCR products on a 4% agarose gel after the reaction to identify contaminants. Take standard precautions to avoid contamination when preparing your PCR reactions. Ideally, amplification reactions should be assembled in a DNA-free environment. We recommend using aerosol-resistant barrier tips.
	Primer dimers or other primer artifacts are present	Use melting curve analysis to identify primer dimers. We recommend using validated pre-designed primer sets or design primers using dedicated software programs or primer databases. Primer contamination or truncated or degraded primers can lead to artifacts. Check the purity of your primers by gel electrophoresis.
No amplification curve appears on the qPCR graph	There is no PCR product	Run the reaction on a gel to determine whether PCR worked. Then proceed to the troubleshooting steps below.
No PCR product is evident, either in the qPCR graph or on a gel	The protocol was not followed correctly	Verify that all steps have been followed and the correct reagents, dilutions, volumes, and cycling parameters have been used.
	Template contains inhibitors, nucleases, or proteases, or has otherwise been degraded.	Purify or re-purify your template.
	Primer design is suboptimal	Verify your primer selection. We recommend using validated pre-designed primers or design primers using dedicated software programs or primer databases.
PCR product is evident in the gel, but not on the qPCR graph	qPCR instrument settings are incorrect	Confirm that you are using the correct instrument settings (dye selection and calibration, reference dye, filters, acquisition points, etc.).
	Problems with your specific qPCR instrument	See your instrument manual for tips and troubleshooting.
PCR efficiency is above 110%	Template contains inhibitors, nucleases, or proteases, or has otherwise been degraded.	Purify or re-purify your template. Inhibitors in the template may result in changes in PCR efficiency between dilutions
	Nonspecific products may be amplified.	Use melting curve analysis if possible, and/or run the PCR products on a 4% agarose gel after the reaction to identify contaminants. Suboptimal primer design may lead to nonspecific products. Use validated pre-designed primers or design primers using dedicated software programs or primer databases.
PCR efficiency is below 90%	The PCR conditions are suboptimal	Verify that the reagents you are using have not been freeze-thawed multiple times and have not remained at room temperature for too long. Verify that the amount of primers you are using is correct.

References

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