SYBR® GreenER™ qPCR SuperMix Universal

Cat. no. 11762-100 **Size: 100 reactions**
Cat. no. 11762-500 **Size: 500 reactions**

Store at 4°C

Description

SYBR® GreenER™ qPCR SuperMix Universal is a ready-to-use cocktail containing all components, except primers and template, for the amplification and detection of DNA in real-time quantitative PCR (qPCR). 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 Universal is supplied at a 2X concentration and contains hot-start *Taq* DNA polymerase, SYBR® GreenER™ fluorescent 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 (1, 2).
- 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 (3).
- UDG and dUTP in the SuperMix prevent the reamplification of carryover PCR products between reactions (4). 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 (5). UDG is then inactivated by the high temperatures during normal PCR cycling, thereby allowing the amplification of genuine target sequences.
- ROX Reference Dye is included as a separate component to normalize the fluorescent signal between reactions for 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.

Component	100-rxn Kit	500-rxn Kit
SYBR® GreenER™ qPCR SuperMix Universal	2 × 1.25 ml	12.5 ml
ROX Reference Dye	100 µl	500 µl

Shipping and Storage

Kit components are shipped on dry ice and should be stored at 4ºC. Storage at –20ºC may extend shelf life.

Handling Conditions

Minimize exposure of both the SuperMix and ROX Reference Dye 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 Universal, catalog nos. 11765-100 and 11765-500.

Additional Products

Product	Amount	Catalog No.
Custom primers	Visit www.invitrogen.com/oligos	
SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR	50 rxns	11752-050
	250 rxns	11752-250
SYBR® GreenER™ Two-Step qRT-PCR Kit Universal	100 PCRs	11765-100
	500 PCRs	11765-500
SYBR® GreenER™ qPCR SuperMix for ABI PRISM®	100 rxns	11760-100
	500 rxns	11760-500
SYBR® GreenER™ qPCR SuperMix for iCycler®	100 rxns	11761-100
	500 rxns	11761-500
Fluorescein NIST-Traceable Standard (50 µM)	5 × 1 ml	F36915

Important Parameters

Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the ABI PRISM® 7000, 7700, and 7900HT; the ABI 7300 and 7500 Real-Time PCR Systems; the ABI GeneAmp® 5700; the Bio-Rad iCycler®; the Stratagene Mx3000P®, Mx3005P™, and Mx4000®; the Corbett Research Rotor-Gene™; the MJ Research DNA Engine Opticon™, Opticon® 2, and Chromo 4™ Real-Time Detector; and the Cepheid Smart Cycler®. Optimal cycling conditions will vary with different instruments.

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 1).
- 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.invitrogen.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.

ROX Reference Dye

ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal for instruments that are compatible with that option. ROX is supplied at a 25 µM concentration, and is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester in 20 mM Tris-HCl (pH 8.4), 0.1 mM EDTA, and 0.01% Tween® 20. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 50-µl reaction	Final ROX Concentration
ABI 7000, 7300 7700, and 7900HT	1.0 µl	500 nM
ABI 7500; Stratagene Mx3000™, Mx3005P™, and Mx4000™	0.1 µl*	50 nM

*To accurately pipet 0.1 µl per reaction, we recommend that you dilute ROX 1:10 immediately before use and use 1 µl of the dilution.

Note: SYBR® GreenER™ qPCR SuperMix for ABI PRISM® includes ROX in the SuperMix at a 500 nM final concentration (see **Additional Products**, page 1)

Fluorescein

The Bio-Rad iCycler® requires the use of fluorescein as a reference dye to normalize the fluorescent reporter signal in reactions with SYBR® GreenER™ qPCR SuperMix. Fluorescein NIST-Traceable Standard is available separately from Invitrogen as a 50-µM solution (see **Additional Products**, page 1). We recommend using a final concentration of 50 nM as a general starting point in qPCR. Optimal results may require a titration between 10 and 100 nM.

SYBR® GreenER™ qPCR SuperMix for iCycler® includes fluorescein in the SuperMix at an optimized concentration for the iCycler® (see **Additional Products**, page 1).

General Protocol for ABI Instruments

Follow the general protocol below for qPCR on ABI real-time instruments. Note the lower amount of ROX Reference Dye required for the ABI 7500. This generic protocol may also be used as a starting point for other real-time instruments.

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 Universal	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.
ROX Reference Dye (optional)	1 µl/0.1 µl	See table on page 2
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.

References

1. Wittwer C.T., Herrmann M.G., Moss A.A., and Rasmussen R.P. (1997) Continuous fluorescence monitoring of rapid cycle DNA amplification. *BioTechniques 22*, 130-138.
2. Ishiguro, T., Saitoh, J., Yawata, H., Yamagishi, H., Iwasaki, S., and Mitoma, Y. (1995) Homogeneous quantitative assay of hepatitis C virus RNA by polymerase chain reaction in the presence of a fluorescent intercalater. *Anal. Biochem. 229*, 207.
3. Chou, Q., Russell, M., Birch, D., Raymond, J., and Bloch, W. (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucl. Acids Res. 20*, 1717.
4. Longo, M., Berninger, M., and Hartley, J. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene 93*, 125.
5. Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B., and Sperens, B. (1977) DNA N-glycosidases: properties of uracil-DNA glycosidase from *Escherichia coli*. *J. Biol. Chem. 252*, 3286.

Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

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.