invitrogen™

Platinum® SYBR® Green qPCR SuperMix-UDG with ROX

Cat. no. 11744-100 **Size: 100 reactions**
Cat. no. 11744-500 **Size: 500 reactions**
Store at –20°C

Description

Platinum® SYBR® Green qPCR SuperMix-UDG with ROX is a ready-to-use reaction mix for the amplification and detection of DNA in real-time quantitative PCR (qPCR) on instruments that support normalization with ROX Reference Dye. It combines the automatic "hot-start" technology of Platinum® *Taq* DNA polymerase with integrated UDG carryover prevention technology and SYBR® Green I 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.

Platinum® SYBR® Green qPCR SuperMix-UDG with ROX is supplied at a 2X concentration and contains Platinum® *Taq* DNA polymerase, SYBR® Green I dye, Tris-HCl, KCl, 6 mM $MgCl_2$, 400 µM dGTP, 400 µM dATP, 400 µM dCTP, 800 µM dUTP, uracil DNA glycosylase (UDG), 1 µM ROX Reference Dye, and stabilizers.

- Platinum® *Taq* DNA polymerase is recombinant *Taq* DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the denaturation step in PCR cycling, providing an automatic hot start in PCR for increased sensitivity, specificity, and yield (1, 2).
- UDG and dUTP in the SuperMix prevent the reamplification of carryover PCR products between reactions (3). 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 (4). UDG is then inactivated by the high temperatures during normal PCR cycling, thereby allowing the amplification of genuine target sequences.
- SYBR® Green I is a fluorescent dye that binds directly to double-stranded DNA (dsDNA). In qPCR, as dsDNA accumulates, the dye generates a signal that is proportional to the DNA concentration and that can be detected using real-time qPCR instruments (5, 6). SYBR® Green I in this SuperMix formulation can quantify as few as 10 copies of a target gene in as little as 1 pg of template DNA or RNA. It has a broad dynamic range of six orders of magnitude, and is compatible with melting curve analysis.
- 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.

Magnesium chloride (50 mM) is provided as a separate component to allow adjustment of the magnesium concentration for optimal performance. ROX Reference Dye is included as a separate component to normalize the fluorescent signal between reactions, for instruments that are compatible with this option.

Component	100-rxn Kit	500-rxn Kit
Platinum® SYBR® Green qPCR SuperMix-UDG with ROX	2 × 1.25 ml	12.5 ml
50 mM Magnesium Chloride ($MgCl_2$)	1 ml	2 × 1 ml

Storage

Store components in the dark at -20ºC. Minimize exposure of Platinum® SYBR® Green qPCR SuperMix-UDG with ROX 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 kit is also included with the SuperScript™ Platinum® SYBR® Green Two-Step qRT-PCR Kit with ROX, catalog nos. 11748-100 and 11748-500.

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
Platinum® SYBR® Green qPCR SuperMix-UDG	100 rxns	11733-038
	500 rxns	11733-044
SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit with ROX	100 rxns	11746-100
	500 rxns	11746-500

Important Parameters

Instrument Compatibility

This kit can be used with 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, 7900HT, and 7900HT Fast; 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 and 7500 Fast, and the Stratagene Mx3000P®, Mx3005P™, and Mx4000®. For these instruments, we recommend Platinum® SYBR® Green qPCR SuperMix-UDG, which includes ROX as a separate tube that can be added at the required concentration (see **Additional Products**, page 1).

Template

cDNA

For two-step qRT-PCR, use 5 µl of undiluted or 10 µl of diluted cDNA generated from 10 pg to 1 µg of total RNA. For cDNA synthesis, we recommend SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR (see **Additional Products**, page 1).

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 100 pg to 1 µg 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 a SYBR® Green detection system. 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. Optimal results may require a titration of primer concentrations between 100 and 500 nM. A final concentration of 200 nM per primer is effective for most reactions.

Melting Curve Analysis

Melting curve analysis should always be performed during real-time qPCR to identify the presence of primer dimers and analyze the specificity of the reaction. For more information, visit www.invitrogen.com/qpcr.

Magnesium Concentration

Magnesium chloride is included in the SuperMix at a final concentration of 3 mM. This works well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the 50-mM magnesium chloride provided in the kit to increase the magnesium concentration, as shown below (the table assumes a 50-µl reaction containing 25 µl of SuperMix):

For a Final $MgCl_2$ Concentration of	Add this Volume of 50-mM $MgCl_2$ (per 50-µl Rxn)
4.0 mM	1 µl
5.0 mM	2 µl
6.0 mM	3 µl

Decrease the amount of water in the reaction accordingly.

Quality Control

This product is tested functionally in real-time qPCR using plasmid DNA. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration over six orders of magnitude. Platinum® SYBR® Green qPCR SuperMix-UDG with ROX is evaluated in a DNA polymerization activity assay that measures the percent of *Taq* DNA polymerase inhibition versus an uninhibited control. The components of Platinum® SYBR® Green qPCR SuperMix-UDG with ROX are tested for the absence of DNase, RNase, and contaminating exonuclease activities. Recombinant *Taq* DNA polymerase is tested for the absence of contaminating exonuclease and double- and single-stranded endonuclease activities. The enzyme is >90% homogeneous as determined by SDS-polyacrylamide gel electrophoresis.

General Protocol for ABI Instruments

Follow the general protocol below for qPCR on compatible ABI real-time instruments (see **Instrument Compatibility**, page 2).

For protocols for specific instruments, visit www.invitrogen.com/qpcr. 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).

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 2 minutes hold
   40 cycles of:
   95ºC, 15 seconds
   60ºC, 30 seconds (60 seconds for the 7900HT)

   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. Volumes for a single 50-µl reaction are listed. 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:** Preparation of a master mix is *crucial* in qPCR to reduce pipetting errors.

Component	Single rxn
Platinum® SYBR® Green qPCR SuperMix-UDG with ROX	25 µl
Forward primer, 10 µM	1 µl
Reverse primer, 10 µM	1 µl
Template (100 pg to 1 µg of genomic DNA, 10–10^7 copies of plasmid DNA, or cDNA generated from 10 pg to 1 µg of total RNA)	≤ 10 µl
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

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3. 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.
4. 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.
5. 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.
6. 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.

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, 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.