# Platinum® Quantitative PCR SuperMix-UDG with ROX

**Cat. No. 11743-100** **Size: 100 reactions**
**Cat. No. 11743-500** **Size: 500 reactions**
**Store at –20°C**

## Description

Platinum® Quantitative PCR 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. The SuperMix combines the automatic "hot-start" technology of Platinum® *Taq* DNA polymerase with integrated UDG carryover prevention technology to provide optimal performance with a variety of qPCR detection technologies, including LUX™ Fluorogenic Primers and TaqMan® probes (1–4). Volumes are provided for 100 or 500 amplification reactions of 50 µl each.

The SuperMix is supplied at a 2X concentration and contains Platinum® *Taq* DNA polymerase, 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 (5, 6).
- UDG and dUTP in the SuperMix prevent the reamplification of carryover PCR products between reactions (7). 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 (8). 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.

Magnesium chloride (50 mM) is provided as a separate component to allow adjustment of the magnesium concentration for optimal performance. For quantification of RNA, this SuperMix can be used in a two-step qRT-PCR procedure following cDNA synthesis with a system such as the SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR (catalog nos. 11752-050 and 11752-250).

**Note:** This kit is designed for use with fluorogenic primers or probes. For a SuperMix containing ROX that uses SYBR® Green I dye, we recommend Platinum® SYBR® Green qPCR SuperMix-UDG with ROX (see **Additional Products**, below).

## Components

| Component | 100-rxn Kit | 500-rxn Kit |
|---|---|---|
| Platinum® Quantitative PCR SuperMix-UDG with ROX | 2 × 1.25 ml | 12.5 ml |
| 50 mM Magnesium Chloride | 1 ml | 2 × 1 ml |

## Storage

Store components in the dark at -20ºC.

## Two-Step qRT-PCR Kits

This kit is also included with the SuperScript® Platinum® Two-Step qRT-PCR Kit with ROX, catalog nos. 11747-100 and 11747-500.

## Related Products

| Product | Amount | Catalog No. |
|---|---|---|
| LUX™ Fluorogenic Primers | Visit www.invitrogen.com/lux to order | |
| SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR | 50 rxns | 11752-050 |
| | 250 rxns | 11752-250 |
| Platinum® Quantitative PCR SuperMix-UDG | 100 rxns | 11730-017 |
| | 500 rxns | 11730-025 |
| Platinum® SYBR® Green qPCR SuperMix-UDG with ROX | 100 rxns | 11744-100 |
| | 500 rxns | 11744-500 |

## Guidelines and 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® Quantitative PCR 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 $9.1 \times 10^{11}$ copies divided by the plasmid size in kilobases.

### 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.

### Multiplexing

In multiplex applications, different reporter dyes are used to label separate primers or probes targeting different genes. For relative expression studies using multiplex PCR, the amount of primer for the reference gene (*e.g.*, β-actin or GAPDH) should be limited to avoid competition with the sample gene. In general, the final concentration of the reference gene primer should be between 25 and 100 nM. A primer titration is recommended for optimal results. For additional optimization guidelines, visit www.invitrogen.com/qpcr.

### Detection Methods

For best results using the following detection systems, the amplicon size should be 80–200 bp.

#### *LUX™ Primers*

LUX™ Primers are fluorogenic primers for qPCR that provide high sensitivity, high specificity, multiplexing capability, and melting curve analysis. LUX™ Primers are available separately from Invitrogen (www.invitrogen.com/lux), and may be designed for specific targets using the D-LUX™ Designer at www.invitrogen.com/dluxdesigner.

A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a primer titration between 100 and 500 nM.

#### *Dual-Labeled Probes*

A final probe concentration of 100 nM is effective for most reactions. The optimal concentration may vary between 50 and 500 nM. PCR primers used with probes should be designed according to standard PCR guidelines. A final concentration of 200 nM per primer is effective for most reactions.

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## General Protocol for ABI Instruments

Follow the protocol below for qPCR using either LUX™ Primers or TaqMan® Probes 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 (LUX™ Primers only): Refer to instrument documentation

2. Set up reactions as specified below. 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.

| **LUX™ Primers Reaction Mix** | |
|---|---|
| Component | Single rxn |
| Platinum® Quantitative PCR SuperMix-UDG with ROX | 25 µl |
| LUX™ labeled primer, 10 µM | 1 µl |
| Unlabeled 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 |

| **TaqMan® Probes Reaction Mix** | |
|---|---|
| Component | Single rxn |
| Platinum® Quantitative PCR SuperMix-UDG with ROX | 25 µl |
| Forward primer, 10 µM | 1 µl |
| Reverse primer, 10 µM | 1 µl |
| Fluorogenic probe, 10 µM | 0.5 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.

## Quality Control

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

## References


1. Crockett, A.O., and Wittwer, C.T. (2001) Fluorescein-labeled oligonucleotides for real-time PCR: using the inherent quenching of deoxyguanosine nucleotides. *Anal. Biochem. 290,* 89–97.
2. Gibson, U. E. M., Heid, C. A., and Williams, P.M. (1996) A novel method for real-time quantitative RT-PCR. *Genome Res. 6*, 99
3. Nazarenko, I., Lowe, B., Darfler, M., Ikonomi, P., Schuster, D., and Rashtchian, A. (2002) Multiplex quantitative PCR using self-quenched primers labeled with a single fluorophore. *Nucl. Acids Res. 30,* e37
4. Nazarenko, I., Pires, R., Lowe, B., Obaidy, M., and Rashtchian, A. (2002) Effect of primary and secondary structure of oligodeoxyribonucleotides on the fluorescent properties of conjugated dyes. *Nucl. Acids Res. 30,* 2089–2095
5. Chou, Q., Russel, 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.
6. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., and Daiss, J.L. (1994) Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction. *BioTechnology 12*, 506.
7. 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.
8. 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.

## 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 if possible, and/or run the PCR products on a 4% agarose gel after the reaction to identify contaminants.<br>To reduce the risk of contamination, take standard precautions when preparing your PCR reactions. |
| | Primer dimers or other primer artifacts are present | Use melting curve analysis to identify primer dimers by their lower melting temperature if possible. Use validated primer sets or design primers/probes using dedicated software programs or primer databases.<br>Check the purity of your primers by gel electrophoresis. If agarose gels are used, we recommend cooling the gels before visualization with intercalating dyes. |
| 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. Use validated primer sets or design primers/probes 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.) for your application. |
| | Problems with your specific qPCR instrument | For instrument-specific tips and troubleshooting, visit www.invitrogen.com/qpcr. |
| 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 |
| PCR efficiency is below 90% | The PCR conditions are suboptimal | Verify that the amount of primers/probe you are using is correct and that the labeled primer or probe has not been exposed to direct light.<br>Verify that the reagents you are using have not been freeze-thawed multiple times and have not remained at room temperature for too long. |
| | Multiplex reactions: Primer concentration may be limiting the rate of the reaction. | Perform a single-plex reaction using the same primers and template to check efficiency. Then determine which primer set should be in limiting concentration. Typically, you should limit the amount of primer for the most abundant gene(s). For additional multiplex troubleshooting tips, visit www.invitrogen.com/qpcr. |