invitrogen™

# Platinum® SYBR® Green qPCR SuperMix-UDG

**Cat. no. 11733-038** **Size: 100 reactions**
**Cat. no. 11733-046** **Size: 500 reactions**

**Store at –20°C**

## Description

Platinum® SYBR® Green qPCR SuperMix-UDG is a ready-to-use cocktail containing all components, except primers, for the amplification and detection of DNA in real-time quantitative PCR (qPCR). 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.

The SuperMix 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), 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.

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. BSA is provided as a separate component for optimization on the Roche LightCycler®.

| Component | 100-rxn Kit | 500-rxn Kit |
|---|---|---|
| Platinum® SYBR® Green qPCR SuperMix-UDG | 2 × 1.25 ml | 12.5 ml |
| 50 mM Magnesium Chloride ($MgCl_2$) | 1 ml | 2 × 1 ml |
| ROX Reference Dye | 100 µl | 500 µl |
| 20X Bovine Serum Albumin (ultrapure, non-acetylated) (1 mg/ml) | 300 µl | 1.3 ml |

## Storage

Components may be stored at either -20ºC or 4ºC. ROX Reference Dye must be stored in the dark.

## Handling Conditions

Minimize exposure of Platinum® SYBR® Green qPCR SuperMix-UDG to direct light. Exposure to direct light for an extended period of time may result in loss of fluorescent signal intensity.

## 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 with ROX | 100 rxns | 11744-100 |
| | 500 rxns | 11744-500 |
| SuperScript™ III Platinum® Two-Step qRT-PCR Kit with SYBR® Green | 100 PCRs | 11735-032 |
| | 500 PCRs | 11735-040 |
| SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit | 100 rxns | 11736-051 |
| | 500 rxns | 11736-059 |
| 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®. For instrument-specific protocols, go to www.invitrogen.com/qpcr. Optimal cycling conditions will vary with different instruments.

### 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.

### 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.

### 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, 7900HT, and 7900HT Fast | 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:** Platinum® SYBR® Green qPCR SuperMix-UDG with ROX (Catalog nos. 11744-100 and 11744-500) 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 SYBR® Green reactions. Fluorescein NIST-Traceable Standard is available 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.

### Bovine Serum Albumin (BSA)

BSA (ultrapure, non-acetylated) is included as a separate tube in each kit for use in LightCycler® reactions. For more information, visit www.invitrogen.com/qpcr.

## General Protocol for ABI Instruments

Follow the general protocol below for qPCR on ABI real-time instruments. Note the separate cycling conditions for the ABI 7500 in Fast Mode, and 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.

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.

| **Standard Cycling Program for ABI Instruments** | **Fast Cycling Program (for the ABI 7500 in Fast Mode)** |
|---|---|
| 50°C for 2 minutes hold (UDG incubation)<br>95°C for 2 minutes hold<br>40 cycles of:<br>95°C, 15 seconds<br>60°C, 30 seconds (60 seconds for the 7900HT)<br>Melting curve analysis: Refer to instrument documentation | Select *Fast Mode* on the Thermal Profile tab<br>50°C for 2 minutes hold (UDG incubation)<br>95°C for 2 minutes hold<br>40 cycles of:<br>95°C, 3 seconds<br>60°C, 30 seconds<br>Melting curve analysis: 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.

| Component | Single rxn |
|---|---|
| Platinum® SYBR® Green qPCR SuperMix-UDG | 25 µl |
| Forward primer, 10 µM | 1 µl |
| Reverse primer, 10 µM | 1 µl |
| ROX Reference Dye (optional) | 1 µl/0.1 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 |

*See the table on page 2 for the amount/concentration of ROX to use for your specific instrument.

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

This product is tested functionally in qPCR using plasmid DNA. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration over six orders of magnitude. Components are also tested for the absence of DNase, RNase, and contaminating exonuclease activities.

## References


1. 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.
2. 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.
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.<br>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.<br>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. |