

# Platinum® SYBR® Green qPCR SuperMix UDG

**Cat. No. 11733-038**

**Cat. No. 11733-046**

**Size: 100 reactions**

**Size: 500 reactions**

**Store at –20°C**

## Description

Platinum® SYBR® Green qPCR SuperMix-UDG is a 2X-concentrated, ready-to-use reaction cocktail containing all components, except primers, for the amplification and detection of DNA in real-time quantitative polymerase chain reaction (qPCR) (1). It contains SYBR® Green I fluorescent dye, Platinum® *Taq* DNA polymerase, Mg<sup>++</sup>, uracil-DNA glycosylase (UDG), proprietary stabilizers, and deoxyribonucleotide triphosphates (dNTPs), with dUTP instead of dTTP. The convenient SuperMix formulation delivers excellent sensitivity in the quantification of target sequences, with a linear dose response over a wide range of target concentrations. The master mix format and integrated UDG carryover protection make this reagent mixture well suited for high-throughput qPCR applications. For quantification of RNA, Platinum® SYBR® Green qPCR SuperMix-UDG can be used in a two-step RT-PCR procedure following cDNA synthesis with a first-strand synthesis kit such as the SuperScript™ III First-Strand Synthesis System for RT-PCR (Cat no. 18080-051).

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 (2, 3). 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.

Platinum® *Taq* DNA polymerase is precomplexed with specific monoclonal antibodies that inhibit *Taq* DNA polymerase activity during reaction assembly at room temperature. Full polymerase activity is restored after the denaturation step in PCR cycling, providing an automatic “hot start” in PCR (4, 5). This significantly reduces nonspecific amplification and mispriming and increases amplification efficiency, sensitivity, and yield (6).

UDG and dUTP are included in the mixture to prevent the reamplification of carryover PCR products between reactions (7, 8, 9). dUTP in the mix ensures that any amplified DNA will contain uracil. UDG, or uracil-N-glycosylase, removes uracil residues from single- or double-stranded DNA, preventing dU-containing DNA from serving as template in future PCRs. Incubation of subsequent PCRs with UDG before cycling destroys any contaminating dU-containing PCR product from previous reactions. After this decontamination step, UDG is inactivated by the high temperatures during normal PCR cycling, thereby allowing the amplification of genuine target sequence(s).

Platinum® SYBR® Green qPCR SuperMix-UDG is supplied at 2X concentration to allow for the addition of primers and template and (if necessary) reference dye and bovine serum albumin (BSA). Magnesium chloride (50 mM) is also 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 LightCycler®. Reagents are provided for 100 or 500 amplification reactions of 50 µl each.

### Component

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 <sub>2</sub> )	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 -20°C or 4°C. ROX Reference Dye must be stored in the dark.

## Platinum® SYBR® Green qPCR SuperMix-UDG (2X)

SYBR® Green I, 60 U / ml Platinum® *Taq* DNA polymerase, 40 mM Tris-HCl (pH 8.4), 100 mM KCl, 6 mM MgCl<sub>2</sub>, 400 µM dGTP, 400 µM dATP, 400 µM dCTP, 400 µM dUTP, 40 U / ml UDG, and stabilizers.

## Quality Control

This product is tested functionally by real-time quantitative analysis of the kanamycin resistance gene using the pCR2.1 plasmid. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration over six orders of magnitude. Platinum® SYBR® Green qPCR SuperMix-UDG is evaluated in a DNA polymerization activity assay that measures the percent of *Taq* DNA polymerase inhibition versus an uninhibited control. Components of Platinum® SYBR® Green qPCR SuperMix-UDG 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.

## Important Parameters

### Template

The target template for real-time qPCR is plasmid DNA (10 to 10<sup>7</sup> copies), genomic DNA (100 pg to 1 µg), or cDNA (generated from 1 pg to 100 ng of total RNA). For best results, the amplicon size should be limited to 80–250 bp in size.

### Primers

Primer selection is one of the most important parameters for qPCR when using a SYBR® Green detection system. To design primers, we strongly recommend using a primer design software program such as OligoPerfect™, available on the Web at [www.invitrogen.com/oligos](http://www.invitrogen.com/oligos). In Step 1 of the OligoPerfect™ designer, you enter your target sequence and select *PCR: Detection* from the Application pulldown menu to access the appropriate design parameters for qPCR applications. Vector NTI™ from InforMax® can also be used to design primers. Using primer design software will ensure that primers are specific for the target sequence and free of internal secondary structure, and avoid complementation at 3'-ends within each primer and with each other.

When designing primers, keep in mind that the amplicon length should be approximately 80–250 bp to optimize the efficiency of qPCR.

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 should always be performed during real-time qPCR to analyze and verify the specificity of the reaction.

**Note on primer dimers:** Melting curve analysis can identify the presence of primer dimers by their lower annealing temperature, compared to the amplicon. The presence of primer dimers is not desirable in samples that contain template, as it decreases PCR efficiency and obscures analysis and determination of cycle thresholds. The formation of primer dimers most often occurs in no-template controls, where the polymerase enzyme is essentially idle, and in this case the quantitative analysis of the template samples is not affected. Melting curve analysis of no-template controls can discriminate between primer dimers and spurious amplification due to contaminating nucleic acids in reagent components.

### Magnesium Concentration

Platinum® SYBR® Green qPCR SuperMix-UDG includes magnesium chloride at a final concentration of 3 mM. Optimal performance for any given target may require adjusting this level of magnesium. If necessary, use the 50-mM magnesium chloride solution included in the kit to increase the magnesium concentration.

Use the following table to determine the amount of additional MgCl<sub>2</sub> to add to achieve a specified concentration (in a 50-µl PCR containing 25 µl of Platinum® SYBR® Green qPCR SuperMix-UDG):

For a Final MgCl <sub>2</sub> Concentration of	Add this Volume of 50-mM MgCl <sub>2</sub> (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.

### Instrument Settings

Platinum® SYBR® Green qPCR SuperMix-UDG can be used with a variety of real-time instruments, including the ABI PRISM® 7700/7000/7900 and GeneAmp® 5700, Bio-Rad iCycler™, Stratagene Mx4000™ and Mx3000P™, Corbett Research Rotor-Gene™, MJ Research DNA Engine Opticon™, Cepheid Smart Cycler®, and Roche LightCycler®. Optimal cycling conditions will vary; refer to your instrument manual for operating instructions. Separate protocols are provided on page 3 for the LightCycler® and instruments that use PCR tubes and/or plates (all other instruments listed above).

### ROX Reference Dye

ROX Reference Dye is included in each kit to normalize the fluorescent reporter signal in real-time qPCR for instruments that are compatible with this option. Its use is optional. ROX Reference Dye can be used to 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 (25 µM) in 20 mM Tris-HCl (pH 8.4), 0.1 mM EDTA, and 0.01% Tween® 20.

ROX is supplied at 50X concentration. Add 1 µl of ROX for every 50 µl of reaction volume. For convenience and to reduce pipetting errors, you can premix a solution of ROX and Platinum® Quantitative PCR SuperMix-UDG. To prepare a master mix:

1. Add ROX Reference Dye to Platinum® Quantitative PCR SuperMix-UDG, at a ratio of 1 µl of ROX for every 25 µl of SuperMix-UDG.
2. Mix by vortexing for 10 seconds.
3. Store mixture at either –20°C or 4°C in the dark. Use 26 µl of a ROX/SuperMix-UDG mixture per 50-µl of real-time PCR volume.

**Note:** Use of ROX Reference Dye is not supported on the LightCycler®. The iCycler™ typically uses fluorescein as the reference dye; see the iCycler™ manual for more information.

### Bovine Serum Albumin (BSA)

BSA (ultrapure, non-acetylated) is included as a separate tube in each kit for use in LightCycler® reactions.

### Handling Conditions

We recommend that you 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.

## Cycling Programs and Protocols

Since PCR is a powerful technique capable of amplifying trace amounts of DNA, all appropriate precautions should be taken to avoid cross-contamination. The tables below provide separate cycling conditions and reaction components for real-time instruments that use PCR tubes/plates (e.g., ABI PRISM<sup>®</sup>, Bio-Rad iCycler<sup>™</sup>, Stratagene Mx4000<sup>™</sup>, Cepheid Smart Cycler<sup>®</sup>) and the Roche LightCycler<sup>®</sup>. After programming the instrument and preparing the reaction mix, follow the steps below the tables to perform the reaction.

**Note:** These cycling conditions are recommended as a starting point and guideline. Optimal cycling temperatures and times may vary for different target sequences, primer sets, and instruments.

Instruments with PCR Tubes/Plates		
<b>Cycling Program</b>		
<u>3-Step Cycling</u>	<u>2-Step Cycling</u>	
50°C for 2 min hold	50°C for 2 min hold	
95°C for 2 min hold	95°C for 2 min hold	
45 cycles of:	50 cycles of:	
95°C, 15 s	95°C, 15 s	
55°C, 30 s	60°C, 30 s	
72°C, 30 s		
<u>Melting Curve Analysis</u>		
Refer to instrument documentation		
<b>Master Mix</b>		
<u>Component</u>	<u>1 rxn</u>	<u>50 rxns</u>
Platinum <sup>®</sup> SYBR <sup>®</sup> Green qPCR SuperMix-UDG <sup>1</sup>	25 µl	1250 µl
ROX Reference Dye (optional)	1 µl	50 µl
Forward primer, 10 µM	1 µl	50 µl
Reverse primer, 10 µM	1 µl	50 µl
Autoclaved distilled water	to 40 µl	to 2000 µl
<b>Protocol</b>		
<ol style="list-style-type: none"> <li>1. Program the real-time instrument to perform a brief UDG incubation immediately followed by PCR amplification, as shown in the cycling programs above. Optimal cycling temperatures and times may vary for different target sequences, primer sets, and instruments.</li> <li>2. Prepare a master mix of all components except template as specified above. <b>Note:</b> Preparation of a master mix is <b>crucial</b> in quantitative applications to reduce pipetting errors.</li> <li>3. For each reaction, add 40 µl of the master mix to a 0.2-ml microcentrifuge tube or each well of a 96-well PCR plate.</li> <li>4. Add 10 µl of sample template—plasmid DNA (10 to 10<sup>7</sup> copies), genomic DNA (100 pg to 1 µg), or cDNA (from 1 pg to 100 ng of total RNA)—to each reaction vessel and cap or seal the tube/plate.</li> <li>5. Gently mix and make sure that all components are at the bottom of the reaction vessel. Centrifuge briefly if needed.</li> <li>6. Place reactions in a thermal cycler programmed as described above. Collect and analyze the results.</li> </ol>		

LightCycler <sup>®</sup>		
<b>Cycling Program</b>		
<u>PCR Cycling</u>		
<i>Program choice:</i> Amplification		
<i>Analysis mode:</i> Quantification		
50°C for 2 min hold		
95°C for 2 min hold		
45 cycles of:		
94°C, 5 s		
55°C, 10 s (single acquire)		
72°C, 10 s		
<u>Melting Curve Analysis</u>		
<i>Program choice:</i> Melting curve		
<i>Analysis mode:</i> Melting curves		
95°C, 0 s		
55°C, 15 s		
95°C, 0 s		
40°C, 0 s		
<b>Master Mix</b>		
<u>Component</u>	<u>1 rxn</u>	<u>34 rxns</u>
Platinum <sup>®</sup> SYBR <sup>®</sup> Green qPCR SuperMix-UDG <sup>1</sup>	10 µl	340 µl
20X Bovine Serum Albumin (1 mg/ml) <sup>2</sup>	1 µl	34 µl
Forward primer, 10 µM	1 µl	34 µl
Reverse primer, 10 µM	1 µl	34 µl
Autoclaved distilled water	to 18 µl	612 µl
<b>Protocol</b>		
<ol style="list-style-type: none"> <li>1. Program the LightCycler<sup>®</sup> to perform a brief UDG incubation immediately followed by PCR amplification, as shown in the cycling programs above. Optimal cycling temperatures and times may vary for different target sequences and primer sets.</li> <li>2. Set the fluorescence on the LightCycler<sup>®</sup> to the F1 channel.</li> <li>3. Prepare a master mix of all components except template as specified above. <b>Note:</b> Preparation of a master mix is <b>crucial</b> in quantitative applications to reduce pipetting errors.</li> <li>4. For each reaction, add 18 µl of the master mix to a capillary tube.</li> <li>5. Add 2 µl of sample template—plasmid DNA (10 to 10<sup>7</sup> copies), genomic DNA (100 pg to 1 µg), or cDNA (from 1 pg to 100 ng of total RNA)—to each capillary tube and cap the tube.</li> <li>6. Centrifuge tubes at 700 × g for 5 seconds.</li> <li>7. Place reaction tubes in the rotor of the LightCycler<sup>®</sup> and run the program. Collect and analyze the results.</li> </ol>		

<sup>1</sup>Final concentration: 1.5 U Platinum<sup>®</sup> Taq DNA polymerase, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3 mM MgCl<sub>2</sub>, 200 µM dGTP, 200 µM dATP, 200 µM dCTP, 400 µM dUTP, 1 U UDG

<sup>2</sup>BSA is required for LightCycler<sup>®</sup> reactions. Validated with non-acetylated ultrapure BSA (10% solution).

## Related Products

<u>Product</u>	<u>Amount</u>	<u>Catalog No.</u>
Custom primers	Visit <a href="http://www.invitrogen.com/oligos">www.invitrogen.com/oligos</a> to order	
SuperScript™ III First-Strand Synthesis System for RT-PCR	50 rxns	18080-051
ROX Reference Dye	500 µl	12223-012

## References

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