Platinum® Quantitative PCR SuperMix-UDG

Cat. no. 11730-017 **Size: 100 reactions**
Cat. no. 11730-025 **Size: 500 reactions**
Store at –20°C

Description

Platinum® Quantitative PCR SuperMix-UDG is a ready-to-use cocktail containing all components, except primers, for the amplification and detection of DNA in real-time qPCR. 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), 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, preventing dU-containing DNA from serving as template in future PCRs (8). A UDG incubation step before PCR cycling destroys any contaminating dU-containing product from previous reactions. UDG is then inactivated by the high temperatures during normal PCR cycling, thereby allowing the amplification of genuine target sequences.

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.

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

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

Storage

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

Quality Control

This product is tested functionally in qPCR using genomic DNA. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration and detection from 10 pg human genomic DNA. Components are also tested for the absence of DNase, RNase, and contaminating exonuclease activities.

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Additional Products

Product	Amount	Catalog No.
SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR	50 rxns	1172-050
	250 rxns	11752-250
Platinum® Quantitative PCR SuperMix-UDG with ROX	100 rxns	11743-100
	500 rxns	11743-500
Platinum® SYBR® Green qPCR SuperMix-UDG	100 rxns	11733-038
	500 rxns	11733-046
LUX™ Fluorogenic Primers	Visit www.invitrogen.com/lux	

Guidelines and 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 9.1×10^{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.

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 (per 50-µl reaction volume):

Instrument	ROX per 50-µl reaction	Final 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 diluting ROX 1:10 immediately before use and use 1 µl of the dilution.

Note: Platinum® Quantitative PCR SuperMix-UDG with ROX (Catalog nos. 11743-100 and 11743-500) includes ROX in the SuperMix at a 500 nM final concentration (see **Additional Products**, page 1)

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.

General Protocol for ABI Instruments

Follow the protocol below for qPCR using either LUX™ Primers or TaqMan® Probes 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 and 7500 Fast systems. This generic protocol may also be used 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) 95°C for 2 minutes hold 40 cycles of: 95°C, 15 seconds 60°C, 30 seconds (60 seconds for the 7900HT) Melting curve analysis (LUX™ Primers only): Refer to instrument documentation	Select *Fast Mode* on the Thermal Profile tab 50°C for 2 minutes hold (UDG incubation) 95°C for 2 minutes hold 40 cycles of: 95°C, 3 seconds 60°C, 30 seconds 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	25 µl
LUX™ labeled primer, 10 µM	1 µl
Unlabeled 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

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

References

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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. 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. 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. 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.