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SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit

Catalog No.	Size/Formulation:	Store at room temperature
11728-081	96-well plate	
11728-089	96-well plate, with ROX Reference Dye	
11728-097	12 × 8 strip wells, plate format	

Description

The SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit provides qualified reagents in lyophilized form for the sensitive and reproducible detection and quantification of RNA in one-step quantitative RT-PCR (qRT-PCR). The one-step reaction mix is aliquoted into plate wells or strip wells and then lyophilized, allowing for room temperature storage, the addition of large sample volumes, and ease of reaction setup. To perform qRT-PCR, simply add water, primers, and template, vortex to dissolve the pellet, and proceed with the reaction.

This kit combines the high-temperature reverse transcription capability of SuperScript™ III RT with the automatic hot-start PCR capability provided by Platinum® *Taq* DNA Polymerase for optimal specificity, consistency, and efficiency. Both cDNA synthesis and qPCR are performed in a single tube using gene-specific primers and RNA target(s) from either total RNA or mRNA. The one-step formulation enables highly sensitive detection from as few as 10 copies of RNA template, with a broad dynamic range that supports accurate quantification of high-copy mRNA at up to 1 µg of total RNA.

Each pellet contains SuperScript™ III RT, Platinum® *Taq* DNA Polymerase, a proprietary buffer system, Mg^{++}, dNTPs, RNaseOUT™ Recombinant Ribonuclease Inhibitor, and stabilizers. The final concentration of dNTPs is 200 nM each and the final concentration of Mg^{++} is 4 mM. Catalog no. 11728-089 also includes ROX Reference Dye in each pellet, to normalize the fluorescent signal between reactions for instruments that are compatible with this option.

SuperScript™ III Reverse Transcriptase is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability (1, 2). The enzyme can be used to synthesize cDNA at a temperature range of 45–60°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Because SuperScript™ III RT is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA.

Platinum® *Taq* DNA polymerase is recombinant *Taq* DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures (3, 4, 5). Activity is restored after the denaturation step in PCR cycling at 94°C, providing an automatic "hot start" in PCR for increased sensitivity, specificity, and yield.

The SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit provides optimal performance for both fluorogenic primer-based detection technology, such as Invitrogen's LUX™ Primers, and fluorogenic hybridization probe-based detection methods such as TaqMan® probes and molecular beacons (6–16). We do not recommend using this kit with dsDNA binding dyes such as SYBR® Green I.

The final volume of each reaction, including primers, template, and water, is 25 µl.

Cat. No.	Product Format	No. of Reactions*	Instrument Recommendation
11728-081	96-well plate	96	ABI PRISM® 7000/7300/7700/7500, Bio-Rad iCycler™, Stratagene Mx3000P™ and Mx4000®, MJ Opticon®
11728-089	96-well plate, with ROX Reference Dye	96	ABI PRISM® 7000/7300/7700/7500 Stratagene Mx3000P™ and Mx4000™, MJ Opticon® 2
11728-097	12 × 8 strip wells, plate format	96	Roche LightCycler®, Corbett Rotor-Gene™, Cepheid Smart Cycler®

*25-µl reaction volume, including primers, template, and water

Quality Control

The product is tested functionally by real-time quantitative analysis using total HeLa RNA as template. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration and detection of β-actin mRNA in 1 pg of total HeLa RNA.

Related Products

Product	Amount	Catalog no.
Micro-to-Midi Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml	15596-026
	200 ml	15596-018
DNase I, Amplification Grade	100 units	18068-015
Custom Primers	to order, visit www.invitrogen.com	

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This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen TECH-LINE℠ 800 955 6288

Recommendations and Guidelines

Plate Formats

SuperScript™ Platinum® RTS One-Step qRT-PCR reaction mix comes lyophilized in a 96-well microtiter plate or 12 × 8 strip wells in plate format.

96-Well Plate

The microtiter plate comes sealed. To open, peel away the foil seal to expose the wells you want to use and add template and primer. Then reseal the wells for thermal cycling using the heat seal tape provided in each kit.

To seal wells for thermal cycling and subsequent storage:

1. Peel away the plastic backing to expose the sticky side of the heat seal tape.
2. Position the tape sticky-side down on the plate, so that all wells are covered. Press down gently and evenly to seal.

Important: Do not use the heat seal tape provided in the kit to cover wells containing unreconstituted pellets, because the pellets will stick to the tape. Wells containing pellets should remain covered with the original foil seal.

The plate can be cut into four sections of 24 wells each (8 wells × 3 rows). Each plate section can then be run in a separate reaction. To separate, carefully cut the foil seal and tabs connecting each section. Store any unused, foil-covered wells in the original resealable pouch with the desiccant pack to ensure dryness.

12 × 8 Strip Wells in Plate Format

Twelve 8-well strips come inserted in a 96-hole plate frame. Push up gently on the bottom of the wells to remove each strip from the frame.

Strip wells are sealed with snap-on strip-caps. Pull up gently on the end of a row of caps to unsnap them. To reseal the plates for thermal cycling and storage, snap the caps back into place. Store any unused strips in the original resealable pouch with the desiccant pack to ensure dryness.

Note: Only strip wells are compatible with the ABI PRISM® 7900. Strip wells require the use of the MicroAmp 96-well tray/retainer set (ABI catalog number 403081).

RNA

- High-quality, intact RNA is essential for full-length, high-quality cDNA synthesis and accurate mRNA quantification.
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained. The lyophilized reaction mix includes RNaseOUT™ to protect against RNA degradation.
- To isolate total RNA, we recommend the Micro-to-Midi Total RNA Purification System (Cat. no. 12183-018) or TRIzol® Reagent (Cat. Nos. 15596-026/-018). Oligo(dT)-selection for poly(A)$^+$ RNA is not necessary, although incorporating this step may improve the yield of specific cDNAs.
- RNA may be treated with DNase I (Catalog no. 18068-015) to remove any contaminating genomic DNA.

Magnesium Concentration

Magnesium is included at a final concentration of 4 mM, which is appropriate for most targets.

ROX Reference Dye

Catalog no. 11728-089 includes ROX Reference Dye in the pellet to normalize the fluorescent reporter signal in qPCR for instruments that are compatible with this option. Note that the use of ROX Reference Dye is **not supported** on the iCycler™, Rotor-Gene™, Opticon®, and LightCycler® platforms.

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.

Instrument Settings

This kit can be used with a variety of qPCR instruments, including but not limited to the ABI PRISM® 7000/7300/7500/7700/7900 and GeneAmp® 5700, the Bio-Rad iCycler™, the Stratagene Mx3000P™ and Mx4000®, the Corbett Research Rotor-Gene™, the MJ Research DNA Engine Opticon® and Opticon® 2, the Cepheid Smart Cycler®, and the Roche LightCycler®. Please refer to your instrument user manual for operating instructions. Optimal cycling conditions will vary with different machines. The protocols on the following page have been optimized for the ABI PRISM® 7700 and the Roche LightCycler®.

Primers

- Gene-specific primers (GSP) are required. We do not recommend using oligo(dT) or random primers, which may generate nonspecific products in the one-step procedure and reduce the amount of product.
- Primers should be designed according to standard PCR guidelines. We recommend using primer design software such as OligoPerfect™ Designer (www.invitrogen.com/oligoperfect). LUX™ Primers must be designed using LUX™ Designer (www.invitrogen.com/lux).
- Primers should be specific for the target sequences, be free of internal secondary structure, and should avoid complementation at 3′ end within each primer, primer pair, or hybridization probe sequence (except as required for hairpin primers such as LUX™). For best results, the amplicon size should be limited to 80–200 bp.
- Design primers that anneal to exons on both sides of an intron or within the exon/exon boundary of the mRNA to allow differentiation between amplification of cDNA and potential contaminating genomic DNA.
- Optimal results may require a primer titration between 100 and 500 nM. A final concentration of 200 nM per primer is effective for most reactions.
- For multiplex applications, limit the amount of primer for the reference gene, such as β-actin or GAPDH, to avoid competition between amplification of the reference gene and sample gene. In general, the final concentration of the reference gene primer should be between 25 and 100 nM. However, a primer titration is recommended for optimal results.

Dual-Labeled Probes

- The optimal concentration of probe may vary between 50 and 800 nM. See the protocol on the next page for a recommended starting concentration.
- The probe sequence should be free of secondary structure and should not hybridize to itself or to primer 3′ ends.
- For multiplex applications, the concentration of each probe may need to be adjusted independently to obtain optimal fluorescent signals. The amount of probe for the reference gene, such as β-actin or GAPDH, should be limited as described above for primers.

Reaction Setup and Conditions

- Efficient cDNA synthesis can be accomplished in a 5–30 min incubation at 45–60°C. Optimal temperature varies for different primers and templates. A good starting point is 50°C for 10 minutes. For problematic templates, or to increase the specificity of cDNA priming, increase the cDNA synthesis temperature to 55°C.
- SuperScript™ III RT is inactivated, the RNA/cDNA hybrid is denatured, and Platinum® *Taq* DNA polymerase is activated during the 2-min incubation at 95°C.
- The annealing temperature should be 0–10°C below the melting temperature of the primers used.
- The extension time of 30 seconds for instruments that use PCR tubes/plates is appropriate for the short amplicons that are typically used in qPCR.

Quantitative One-Step RT-PCR Protocol

The tables below show the cycling conditions for one-step qRT-PCR using either LUX™ Primers or dual-labeled probes. Separate cycling programs and protocols are provided for instruments that use PCR tubes/plates (e.g., ABI PRISM®, Bio-Rad iCycler™, Stratagene Mx4000™, Cepheid Smart Cycler®) and the Roche LightCycler®. After programming your instrument, follow the steps at the bottom of the page to perform the reaction.

LUX™ Primers	
Cycling Program	
Instruments using PCR tubes/plates	LightCycler®
42–55°C for 10 min hold*	*Program choice:* Amplification
95°C for 2 min hold	*Analysis mode:* Quantification
40–50 cycles of:	42–55°C for 10 min hold*
95°C, 15 s	95°C for 2 min hold
60°C, 30 s	50 cycles of:
	95ºC, 5 s
	60ºC, 20 s (single acquire)
Melting Curve Analysis:	Melting Curve Analysis:
Refer to instrument documentation	*Program choice:* Melting curve
	Analysis mode: Melting curves
	95ºC, 0 s
	55ºC, 15 s
*Use 50°C for 10 min as a general starting point.	95ºC, 0 s
	40ºC, 0 s

Dual-Labeled Probes	
Cycling Program	
Instruments using PCR tubes/plates	LightCycler®
42–55°C for 10 min hold*	*Program choice:* Amplification
95°C for 2 min hold	*Analysis mode:* Quantification
40–50 cycles of:	42–55°C for 10 min hold*
95°C, 15 s	95°C for 2 min hold
60°C, 30 s	50 cycles of:
	95ºC, 5 s
	60ºC, 20 s (single acquire)
Melting curve analysis is possible with some types of dual-labeled probes but not others; refer to probe documentation	
*Use 50°C for 10 min as a general starting point.	

Protocol for Instruments Using PCR Tubes/Plates

1. Program the qPCR instrument to perform cDNA synthesis immediately followed by PCR amplification, as specified above. Optimal temperatures and incubation times may vary for different target sequences (see **Reaction Setup and Conditions**, previous page).
2. Add primers, template, and sterile, distilled water to each well to a final volume of 25 µl, as follows:
   - Amount of template: 1 pg to 1 µg total RNA
   - Recommended final concentration of primers: 200 nM each (e.g., 0.5 µl of a 10 µM stock)
   - If you are using TaqMan® probes, recommended final concentration of probe: 100 nM (e.g., 0.25 µl of a 10 µM stock)
3. Seal the plates/strip wells (see page 2 for sealing instructions) and vortex for 5–10 seconds. **Note: Vortexing is crucial to ensure complete dissolution of the pellet.** Centrifuge briefly to collect the contents.
4. If your qPCR instrument cannot accommodate the plates or strip wells, transfer each 25-µl reaction to a tube appropriate for use with your qPCR instrument.
5. Place reactions in a thermal cycler programmed as described above. Collect and analyze the results.

Protocol for Roche LightCycler®

1. Program the LightCycler® to perform cDNA synthesis immediately followed by PCR amplification, as specified above. Optimal temperatures and incubation times may vary for different target sequences (see **Reaction Setup and Conditions**, previous page).
2. Set the fluorescence on the LightCycler® to the appropriate channel for the reporter dye used (e.g., F1 channel for FAM).
3. Add primers, template, and sterile, distilled water to each well to a final volume of 25 µl, as follows:
   - Amount of template: 1 pg to 1 µg total RNA
   - Recommended final concentration of primers: 500 nM each (e.g., 1.25 µl of a 10 µM stock)
   - If you are using TaqMan® probes, recommended final concentration of probe: 250 nM (e.g., 0.625 µl of a 10 µM stock)
4. Seal the plates/strip wells (see page 2 for sealing instructions) and vortex for 5–10 seconds. **Note: Vortexing is crucial to ensure complete dissolution of the pellet.** Centrifuge briefly to collect the contents.
5. Unseal/uncap the wells and transfer 20 µl of each reaction to each LightCycler® capillary tube. Cap the capillary tube.
6. Centrifuge tubes at 700 × *g* for 5 seconds.
7. Place reaction tubes in the rotor of the LightCycler® and run the program. Collect and analyze the results.

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LightCycler® is a registered trademark of Idaho Technologies, Inc.

Troubleshooting Guide

Problem	Possible Cause	Probable Solution
No amplification product Relative fluorescent signal ≤ background or no template control	Problem with reporter dye or instrument settings	Run the sample on a gel to determine if the PCR product was generated. If the appropriate bands are seen, see "Reporter dye not functional" under Possible Cause or check the instrument settings.
	cDNA synthesis temperature too high, low priming efficiency	Lower incubation temperature.
	RT or cDNA primer blocked by secondary structure	Raise incubation temperature. Redesign primer(s).
	RNA has been damaged or degraded	Replace RNA if necessary.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
	Reporter dye not functional	Validate fluorescent primer or probe design and presence of fluorophore and/or quencher. Redesign and/or resynthesize if necessary.
Poor sensitivity	Not enough starting template RNA	Increase the concentration of template RNA; use 10 ng to 1 μg of total RNA.
Product detected at higher than expected cycle number	RNA has been damaged or degraded	Replace RNA if necessary.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
	RT inhibitors are present in RNA	Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine (19, 20).
	Inefficient cDNA synthesis	Adjust cDNA synthesis temperature and/or primer design.
	Inefficient PCR amplification	Optimize PCR conditions: Adjust annealing temperature as necessary. Increase magnesium concentration. Redesign primers.
Product detected at lower-than-expected cycle number	Too much sample added to reactions	Decrease the concentration of template RNA.
Product detected at lower-than-expected cycle number, and/or positive signal from no-template controls	Template or PCR carry-over contamination	Isolate source of contamination and replace reagent(s). Use separate dedicated pipettors for reaction assembly and post-PCR analysis. Assemble reactions (except for target addition) in a DNA-free area. Use aerosol-resistant pipet tips or positive displacement pipettors.
Unexpected bands after electrophoresis	RNA is contaminated with DNA	Pre-treat RNA with DNase I.
	Oligo(dT) or random primers used	Use gene-specific primers.
	Low specificity in PCR	Optimize PCR conditions as described above.

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