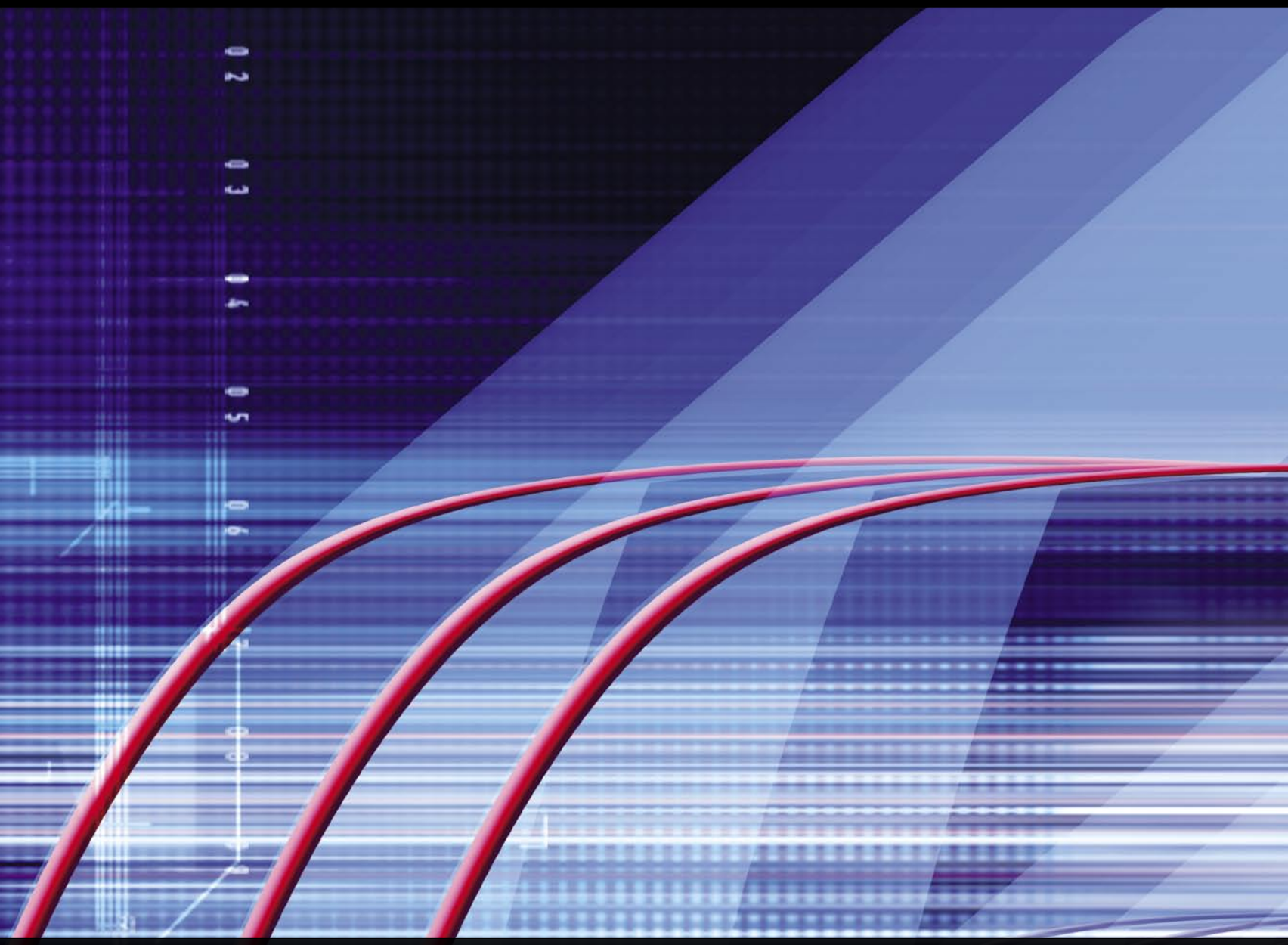


accelerate > qPCR and qRT-PCR performance

accelerate > qPCR and qRT-PCR performance

- Improved qPCR specificity and sensitivity
- Higher efficiency over a broad dynamic range
- Sensitive, specific, and cost-effective detection

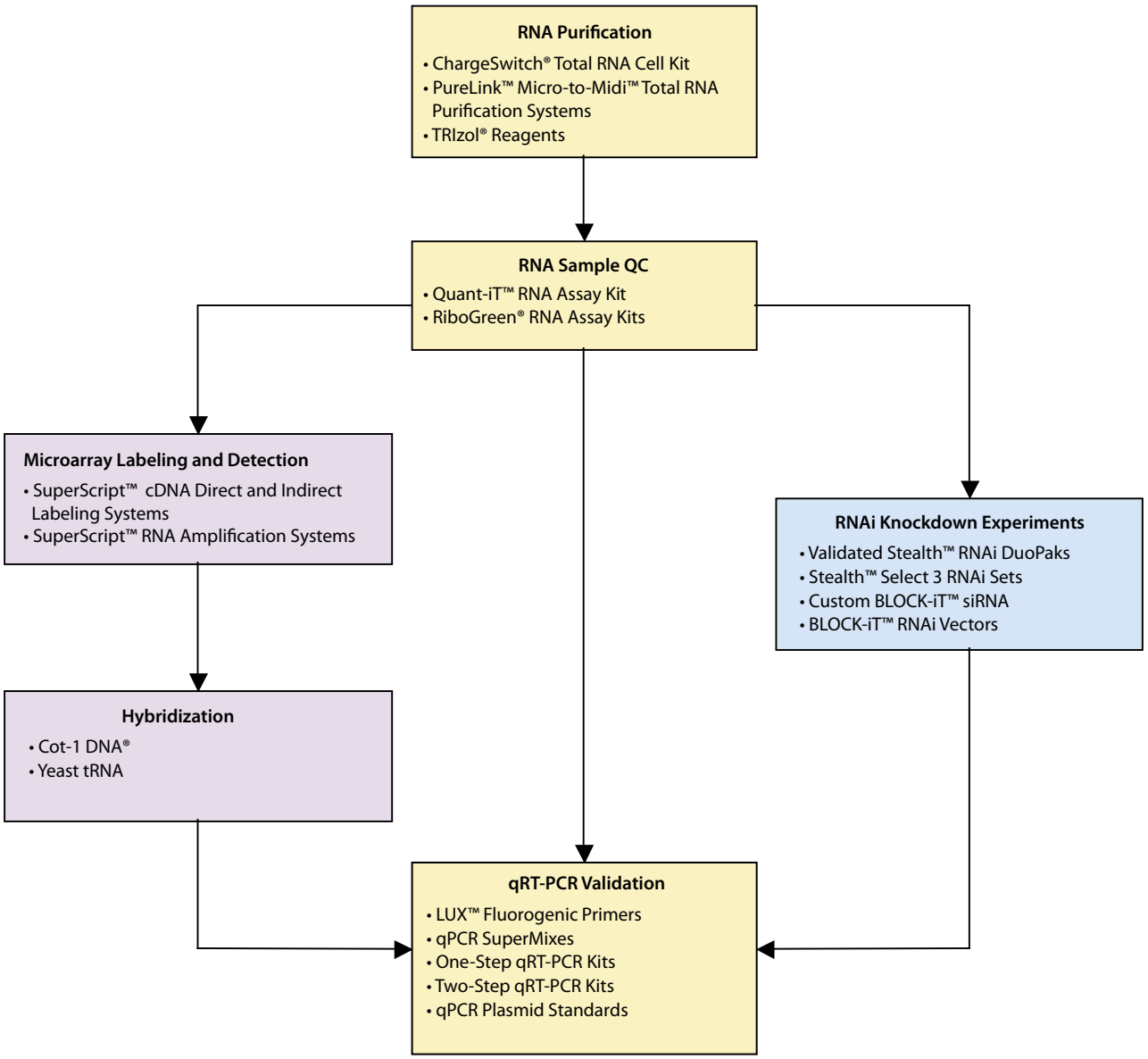
High-performance products for qPCR and qRT-PCR

High-performance research begins with a solid foundation. With Invitrogen and Molecular Probes now under one roof, you can construct your qPCR and qRT-PCR research projects from the ground up with confidence. Invitrogen's superior Platinum® *Taq* and SuperScript™ enzymes combined with Molecular Probes™ detection technologies provide the framework you need to build sensitive, specific qPCR experiments. Our easy-to-use qPCR supermixes, one- and two-step qRT-PCR systems, and fluorescent detection platforms are designed and optimized for unsurpassed performance. In addition, our D-LUX™ fluorescent detection platform offers a powerful and convenient alternative to costly probe-based detection methods. No matter what your challenge, you'll reach new heights in performance and reliability with solid qPCR and qRT-PCR tools from Invitrogen.

Table of contents

Comprehensive solutions for gene expression profiling

From nucleic acid isolation through validation, we have a complete product portfolio to take your gene expression experiments from start to finish.



For ordering information, please see related products on page 30.

Unique technologies set us apart

Our easy-to-use qPCR and qRT-PCR reagent systems and fluorescent detection platforms give you higher sensitivity and greater specificity over a broad dynamic range using the most advanced technologies available:

- Platinum® *Taq* hot-start technology for **improved qPCR specificity and sensitivity**
- SuperScript™ III Reverse Transcriptase (RT) with increased thermostability and a longer half-life for **better sensitivity and the highest cDNA yields**
- Uracil DNA Glycosylase (UDG) for prevention of carryover contamination
- Room Temperature Stable (RTS) technology for **faster, more convenient set-up**
- CellsDirect™ technology for **sensitive qRT-PCR** without a separate RNA purification step
- D-LUX™ Detection Platform for **sensitive, specific, and cost-effective detection**

Two-step vs. one-step qRT-PCR

Two-step qRT-PCR

Two-step qRT-PCR is useful for detecting multiple messages from a single RNA sample, performing multiple PCR amplifications from a single cDNA sample, and /or preserving cDNA for later applications. Two-step qRT-PCR kits are comprised of separate reverse transcriptase and qPCR modules. In Invitrogen's kits, the RT module provides the high-temperature capability of SuperScript™ III RT and an RT reaction mix specifically optimized for first-strand synthesis of cDNA for use in qPCR. The qPCR module includes Platinum® *Taq* DNA polymerase for increased sensitivity, specificity, and yield.

One-step qRT-PCR

One-step qRT-PCR allows easier processing of large numbers of samples from few genes of interest. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and RNA target(s) from either RNA or mRNA. All components necessary for qRT-PCR are mixed in the tube, and reverse transcription is directly followed by PCR without additional handling. Reaction tubes are not opened between cDNA synthesis and amplification so carryover contamination is minimized. Furthermore, by amplifying the entire cDNA sample, one-step qRT-PCR 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 up to 1 µg of total RNA. Table 1 compares the benefits of one-step and two-step qRT-PCR.

Table 1 — Two-step vs. one-step qRT-PCR

	Two-step procedure	One-step procedure
Prime first-strand DNA with:	Oligo(dT) primer Random hexamers	Gene-specific primers
Provides:	**Flexibility**	**Convenience**
	• Choice of primers for cDNA synthesis • Ability to save cDNA for later use •Ability to optimize for difficult qRT-PCR	• Amplification enzymes premixed with reverse transcriptase • Fewer pipetting steps reduce chances for contamination • Well suited for high-throughput applications
Recommended uses:	Ideal for detecting or quantifying multiple genes of interest and/or preservation of cDNA	Ideal for analyzing large numbers of samples from few genes of interest

Select the right qPCR product for you

qPCR and qRT-PCR product selection guide

Use Table 2 to determine the best products for your real-time instrument and application.

Table 2 — qPCR and qRT-PCR product selection guide

		SYBR® Green Detection		Primer/Probe Detection (LUX™ primers or dual-labeled probes)	
Starting material	**Format**	**ABI 7000, 7300, 7700, 7900 instruments**	**Any instrument**	**ABI 7000, 7300, 7700, 7900 instruments**	**Any instrument**
Reagents for qPCR and qRT-PCR					
DNA, cDNA	**qPCR SuperMix, Standard,** *pages 8-9*	Platinum® SYBR® Green qPCR SuperMix-UDG w/ROX	Platinum® SYBR® Green qPCR SuperMix-UDG	Platinum® qPCR SuperMix-UDG w/ROX	Platinum® qPCR SuperMix-UDG
	qPCR SuperMix, Room Temperature Stable, *pages 20-21*	Platinum® RTS SYBR® Green qPCR SuperMix-UDG w/ROX	Platinum® RTS SYBR® Green qPCR SuperMix-UDG	Platinum® RTS qPCR SuperMix-UDG w/ROX	Platinum® RTS qPCR SuperMix-UDG
	qPCR SuperMix, Genotyping, *page 10*	N/A		Platinum® qPCR SuperMix for Genotyping	Platinum® qPCR SuperMix for Genotyping
RNA	**Two-Step Kits, Standard,** *pages 16-17*	SuperScript™ III Platinum® SYBR® Green Two-Step qRT-PCR Kit w/ROX	SuperScript™ III Platinum® SYBR® Green Two-Step qRT-PCR Kit	SuperScript™ III Platinum® Two-Step qRT-PCR Kit w/ROX	SuperScript™ III Platinum® Two-Step qRT-PCR Kit
	Two-Step Kits, Directly from cells, *pages 18-19*	SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit with SYBR® Green	SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit with SYBR® Green	SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit	SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit
	One-Step Kits, Standard, *pages 11-12*	SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit w/ROX	SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit	SuperScript™ III Platinum® One-Step qRT-PCR Kit w/ROX	SuperScript™ III Platinum® One-Step qRT-PCR Kit
	One-Step Kits, Low Abundance Targets, *pages 14-15*			RNA UltraSense™ One-Step qRT-PCR System	RNA UltraSense™ One-Step qRT-PCR System
	One-step Kits, Room Temperature Stable, *page 22*			SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit w/ROX	SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit
Detection systems for qPCR and qRT-PCR					
DNA, cDNA, RNA	**Certified Primer Sets,** *pages 27-28*	N/A		Certified LUX™ Primer Sets for Human Genes	
				Certified LUX™ Primer Sets for Housekeeping Genes	
				Certified LUX™ Primer Sets for Infectious Agents	
	Custom Primer Sets, *pages 23-26*	N/A		D-LUX™ Primer Design Software	
				EvoQuest™ Custom LUX™ Primer Design Service	

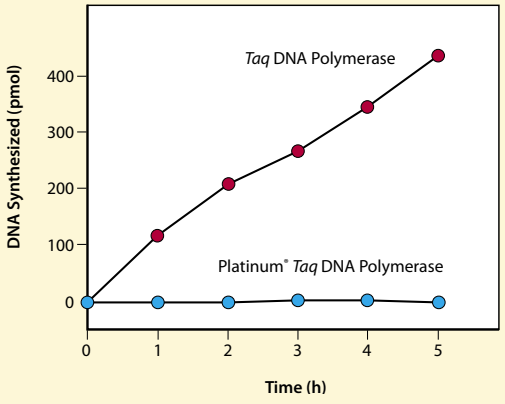
Quality enzymes make all the difference

Platinum® *Taq* hot-start technology improves qPCR sensitivity and specificity

With Platinum® automatic hot-start technology, proprietary antibodies inhibit enzyme activity at low temperatures (Figure 1). During the initial denaturation step, the antibodies are denatured and the enzyme is released into the reaction. Unlike other hot-start technologies that modify *Taq* and can hinder the enzyme's return to full activity, up to 90% of the *Taq* activity in Platinum® *Taq* is restored in just minutes (Figure 2).

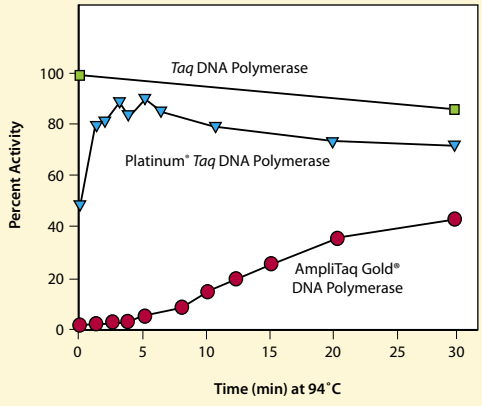
To obtain maximum sensitivity in your qPCR assay, you need the best possible performance with every cycle. Even with long initial activation times, some hot-start polymerases are not fully active during the early cycles. This compromises sensitivity and can mean the difference in detecting a low-copy-number gene. With Platinum® *Taq* you can be sure you achieve high-quality qPCR performance with each and every cycle.

Figure 1 — Complete inhibition of DNA polymerase activity for reaction set-up



Activity assays were run at 37˚C for 5 hours with *Taq* DNA polymerase and Platinum® *Taq* DNA Polymerase.

Figure 2 — Enzyme recovery following thermal activation–Platinum® *Taq* compared to other DNA polymerases



Enzymes were incubated at 94˚C in the reaction buffer provided. Aliquots were removed at the various times and utilized in a standard activity assay.

Uracil DNA Glycosylase (UDG) prevents carryover contamination

As with traditional PCR, qPCR reactions can be affected by carryover contamination of amplification products and primers from previous PCR, leading to false positive results. Uracil DNA glycosylase (UDG) is used prior to the PCR reaction to eliminate carryover and prevent amplification of non-template DNA, reducing false positives and increasing the efficiency of the qPCR reaction.

UDG works by degrading uracil-containing DNA, leaving the natural (thymine containing) target DNA template unaffected. The UDG carryover prevention technique consists of three steps:

1. Incorporation of dUTP in all PCR products by substituting dUTP for dTTP in the reaction mix.
2. Prior to subsequent PCR cycling, the reaction mix is treated with UDG and incubated at 50˚C, allowing the UDG to cleave uracil residues from any contaminating DNA. The removal of the uracil bases causes fragmentation of the DNA, preventing its use as a template for *Taq* DNA polymerase and ensuring no previously amplified products are carried over into the next assay.
3. UDG is thermally inactivated at 95˚C prior to the actual PCR reaction.

UDG and dUTP are included in all of our qPCR and two-step qRT-PCR kits.

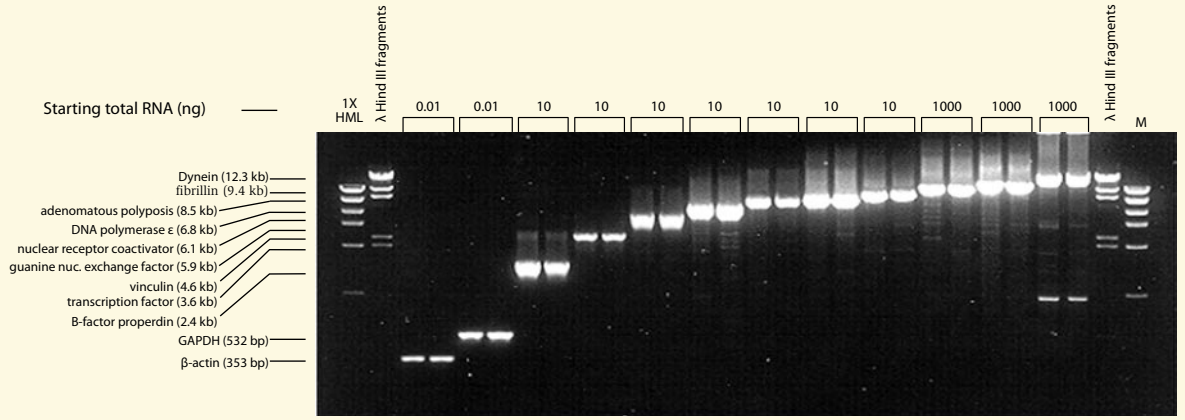
Quality enzymes make all the difference (cont.)

SuperScript™ III RT provides higher thermostability, better qRT-PCR performance

An efficient reverse transcription reaction is critical for generating reliable gene expression data using qPCR. SuperScript™ III RT, a more thermostable mutant of SuperScript™ II RT, is simply the best-performing RT available for your gene expression studies. SuperScript™ III gives you:

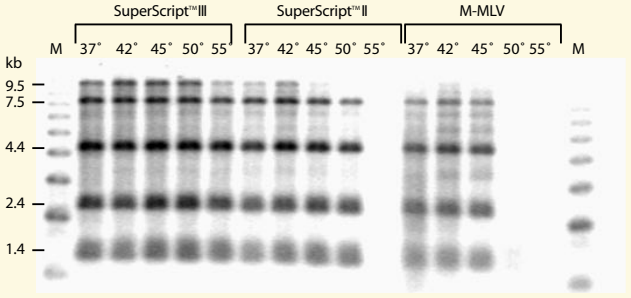
- Reduced RNase H activity for more full-length cDNA (Figure 3)
- A half-life of 220 minutes at 50°C, for the highest cDNA yields (Figure 4)
- Increased thermal stability to 60°C for greater success with GC-rich RNA
- Full activity at 50°C for increased specificity with gene-specific primers

Figure 3 — SuperScript™ III RT generates the highest yield with various-sized targets



cDNA was synthesized from HeLa total RNA or rat total RNA for Dynein with oligo(dT) primer using 400 U of SuperScript™ III RT at 50°C. 10% of cDNA reaction was added to 50-µl PCR reaction containing primers for each gene and 2 U of Platinum® *Taq* DNA Polymerase or 1 U of Platinum® *Taq* DNA Polymerase High Fidelity, 35 or 40 cycles, 1 min/kb.

Figure 4 — SuperScript™ III compared to SuperScript™ II and M-MLV RTs



An autoradiograph is shown of ^{32}P-labeled cDNA synthesized from a mixture of 0.25 µg of RNA each of 1.35 kb, 2.4 kb, 4.4 kb, 7.5 kb, and 9.5 kb with 200 units of each RT at various temperatures. Lane M is ^{32}P-labeled 1 kb DNA ladder.

SuperScript™ III First-Strand Synthesis System for qRT-PCR

SuperScript™ III reverse transcriptase is supplied in our one-step and two-step qRT-PCR kits. For those preferring a modular approach, we offer our SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR, which contains all necessary reagents to generate cDNA. The First-Strand Synthesis SuperMix can be combined with any of our qPCR SuperMixes for sensitive amplification and quantitation.

Product	Quantity	Rxn size	Cat. no.
SuperScript™ III First-Strand Synthesis System for qRT-PCR	50 rxns	20 µl	11752-050
	250 rxns	20 µl	11752-250

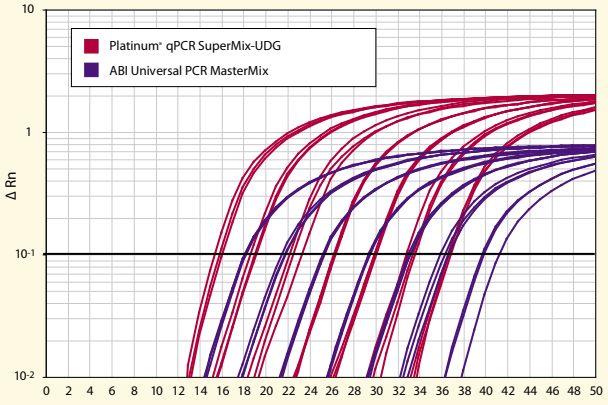
Easy-to-use qPCR SuperMixes

Invitrogen's qPCR SuperMixes are designed to give you sensitive, convenient, and contamination-free qPCR amplification from DNA or cDNA templates. The SuperMix format combines buffer, dNTPs, Platinum® *Taq* DNA Polymerase, and Uracil DNA Glycosylase (UDG) into a ready-to-go mix. You save time by not having to mix your own reagents, while minimizing variation across samples to ensure consistent and reproducible results. Platinum® antibodies inhibit enzyme activity at low temperatures, increasing reaction specificity. In addition, inclusion of 100% dUTP and UDG enzyme provides superior control of carryover contamination, saving time by reducing the number of failed experiments. Platinum® qPCR SuperMix-UDG offers:

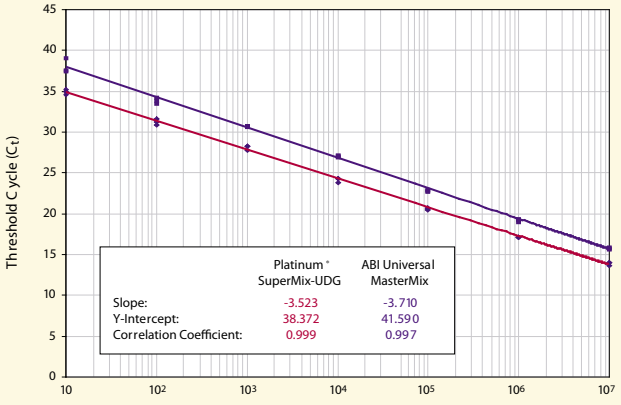
- Superior detection with either LUX™ Fluorogenic Primers or dual-labeled probes (Figure 5)
- The ability to optimize magnesium concentration for different applications and templates
- Choice of a separate tube of ROX Reference Dye for optimization on any instrument, or ROX premixed for ultimate convenience on the ABI 7000, 7300, 7700, and 7900 instruments

Figure 5 — Platinum® qPCR SuperMix-UDG vs. ABI Universal PCR MasterMix

A. Amplification Plot



B. Standard Curve



Panel A: 10 to 10^7 copies of β-actin plasmid DNA were amplified with 200 nM each primer and detected with 100 nM TaqMan® Probe using Platinum® qPCR SuperMix-UDG (red plot) or ABI Universal PCR MasterMix (blue plot). Reactions were assembled at room temperature. PCR incubations were 95°C for 10 min., followed by 50 cycles of 95°C for 15 sec. and 60°C for 1 min. using an ABI PRISM® 7700. **Panel B:** Standard curve showing the starting template amount versus C_t value.

Product	Quantity	Rxn size	Cat. no.
For LUX™ Primers or probe-based detection			
Platinum® qPCR SuperMix-UDG	100 rxns	50 µl	11730-017
(for any instrument)	500 rxns	50 µl	11730-025
Platinum® qPCR SuperMix-UDG with ROX	100 rxns	50 µl	11743-100
(for ABI instruments - 7000, 7300, 7700, 7900)	500 rxns	50 µl	11743-500

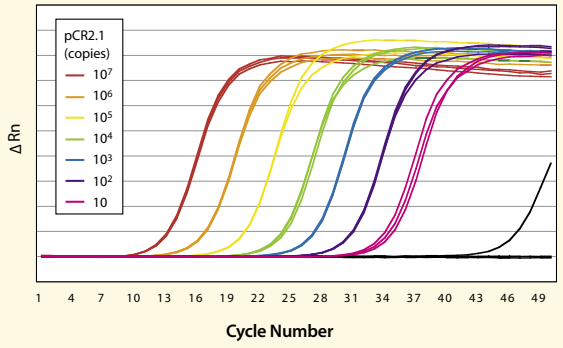
Easy-to-use SYBR® Green qPCR SuperMixes

Platinum® SYBR® Green qPCR SuperMix-UDG includes SYBR® Green I dye for easy, convenient real-time detection. It provides:

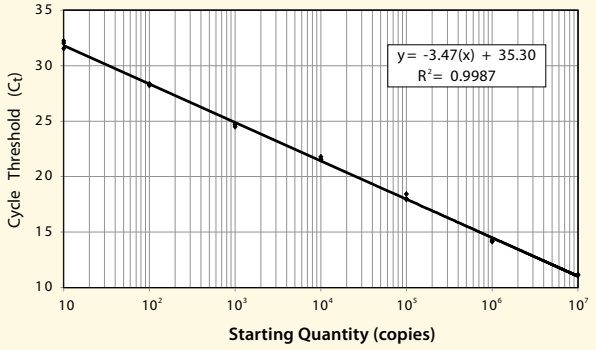
- Sensitive amplification from as few as 10 copies of target and across seven orders of magnitude (Figure 6)
- The ability to optimize magnesium concentration for different applications and templates
- Choice of a separate tube of ROX Reference Dye for optimization on any instrument, or ROX premixed for ultimate convenience with the ABI 7000, 7300, 7700 and 7900, instruments (Figure 7)

Figure 6 — Platinum® SYBR® Green qPCR SuperMix-UDG provides sensitive detection

A. Amplification Plot

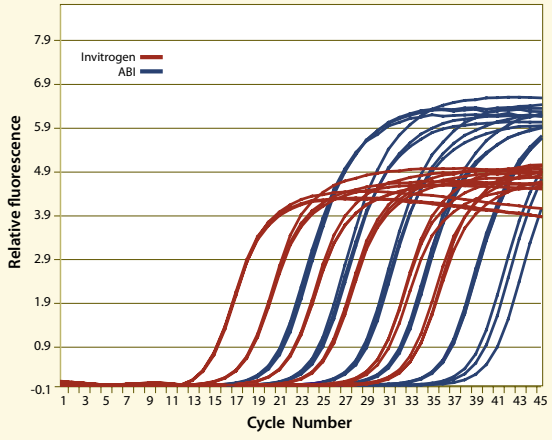


B. Standard Curve



qPCR of 10-fold serial dilutions (10^7 to 10 copies) of pCR®2.1 plasmid was performed using primers specific to the Kanamycin resistance gene (200 nM each) with Platinum® SYBR® Green qPCR SuperMix-UDG and ROX Reference Dye. Reactions were incubated for 2 min. at 50°C, then 2 min. at 95°C, followed by 50 cycles of 95°C for 15 sec.; 60°C, 30 sec. using the ABI PRISM® 7700.

Figure 7 — Invitrogen's Platinum® SYBR® Green qPCR SuperMix-UDG with ROX outperforms ABI's Master Mix



Amplification plots comparing Invitrogen's Platinum® SYBR® Green qPCR SuperMix-UDG w/ROX and ABI's SYBR® Green qPCR MasterMix. Invitrogen's qPCR Plasmid Standard for High Abundance Genes was used as template at concentrations ranging from 10^7 to 10^2 copies were used for detection. Reactions were run on an ABI PRISM® 7700 using ABI's recommended cycling protocol. Even with ABI's protocol, the Invitrogen mix outperformed the ABI mix by over 6 C_ts.

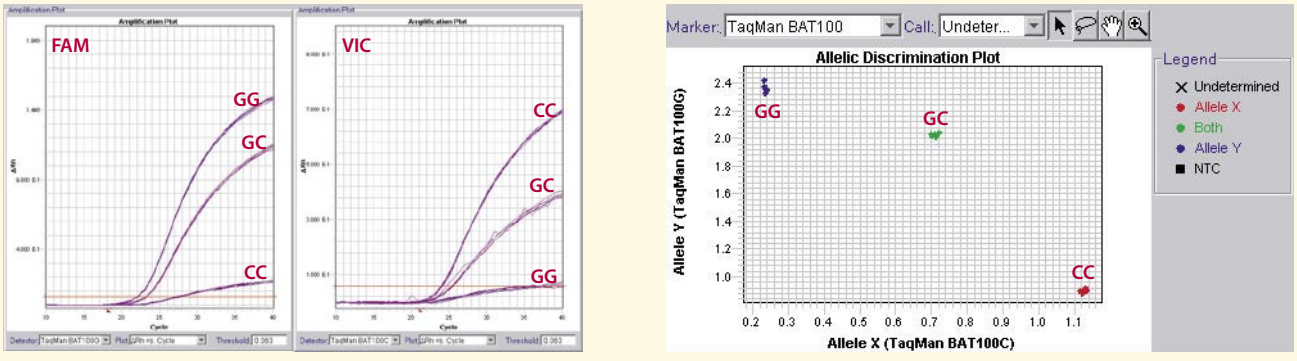
Product	Quantity	Rxn size	Cat. no.
For SYBR® Green-based detection			
Platinum® SYBR® Green qPCR SuperMix-UDG (for any instrument)	100 rxns 500 rxns	50 µl 50 µl	11733-038 11733-046
Platinum® SYBR® Green qPCR SuperMix-UDG with ROX (for ABI instruments - 7000, 7300, 7700, 7900)	100 rxns 500 rxns	50 µl 50 µl	11744-100 11744-500

Easy-to-use qPCR SuperMixes for SNP genotyping

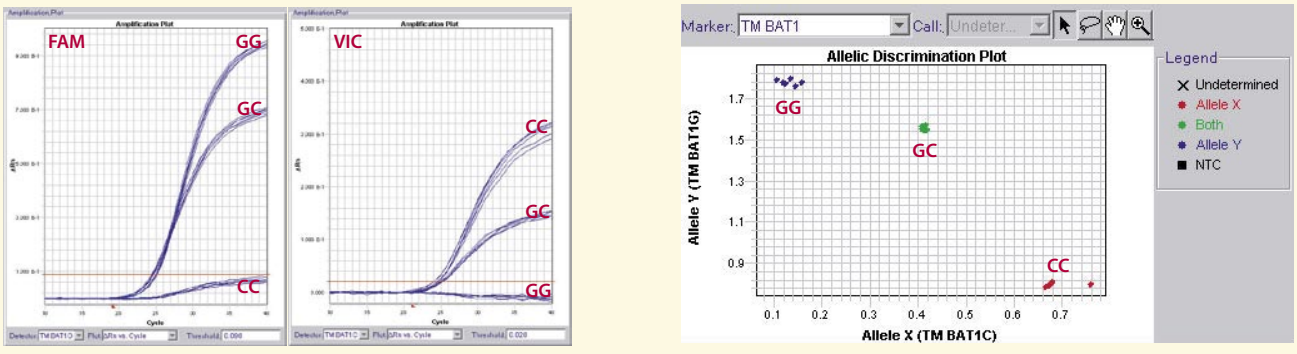
Platinum® qPCR SuperMix-UDG for SNP Genotyping is a ready-to-use reaction mix for the amplification and identification of single-nucleotide polymorphisms (SNPs) in genomic DNA using PCR-based fluorescent SNP genotyping technologies. This SuperMix is specifically formulated for fluorescent discrimination of alleles either by qPCR or by end-point PCR followed by fluorescent plate reader analysis. Special additives provide enhanced fluorescent signals for better discrimination of alleles and excellent separation with minimal scattering between replicate samples (Figure 8). The SuperMix format and integrated UDG carryover prevention make this reagent well-suited for high-throughput applications.

Figure 8 — Platinum® qPCR SuperMix-UDG for SNP Genotyping vs. ABI Universal PCR MasterMix

A. Invitrogen



B. ABI



Panel A. qPCR genotyping results using TaqMan® SNP Genotyping Assay for BAT 1 and Invitrogen's Platinum® qPCR SuperMix-UDG for SNP Genotyping. **Panel B.** qPCR genotyping results using TaqMan® SNP Genotyping Assay for BAT 1 and ABI's Universal Master Mix. Each assay was run according to the reagent manufacturer's recommended protocol on an ABI PRISM® 7900HT.

Product	Quantity	Rxn size	Cat. no.
Platinum® qPCR SuperMix-UDG for SNP Genotyping	250 rxns	20 µl	11729-008
(for any instrument)	1250 rxns	20 µl	11729-016

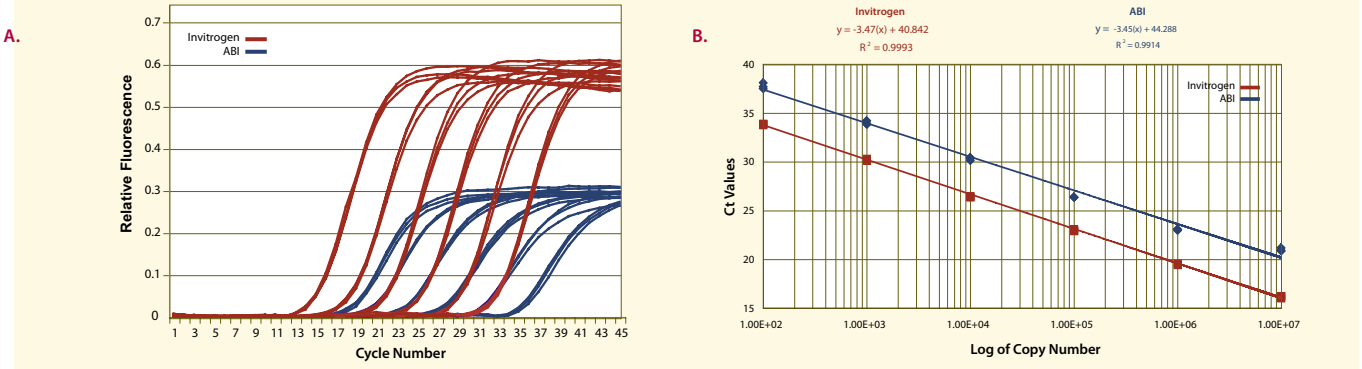
Sensitive one-step qRT-PCR with SuperScript™ III RT

Invitrogen's one-step qRT-PCR systems combine the most powerful RT and DNA polymerase technologies to deliver sensitive qRT-PCR results. The one-step format provides speed, convenience, and less reaction-to-reaction variability.

The SuperScript™ III Platinum® One-Step qRT-PCR Kits are available for use with fluorogenic primers and probes, or with SYBR® Green I dye. Both kits combine the high cDNA yields of SuperScript™ III RT with the hot-start specificity of Platinum® *Taq* DNA Polymerase for precise and accurate analysis in a convenient one-step format. SuperScript™ III Platinum® One-Step qRT-PCR Kits give you:

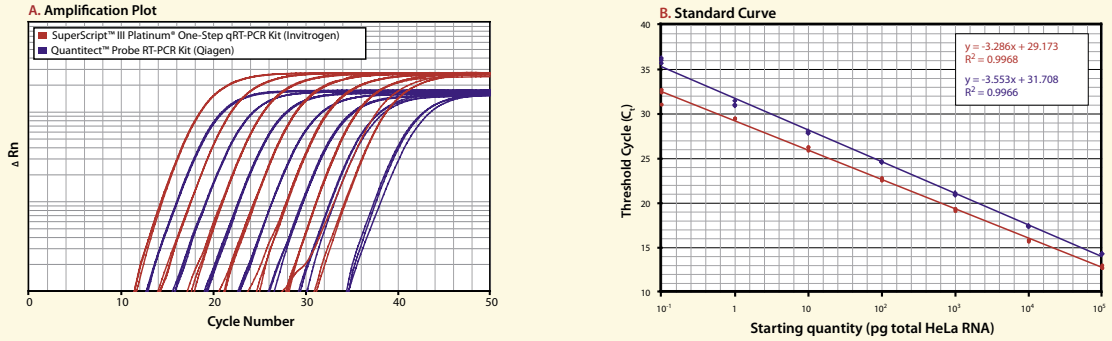
- SuperScript™ III RT for higher temperature cDNA synthesis and greater success with difficult RNA secondary structure
- Platinum® *Taq* DNA Polymerase with hot-start technology for improved specificity
- Superior performance with LUX™ Fluorogenic Primers or dual-labeled fluorogenic probes (Figures 9 and 10)
- SYBR® Green I dye for easy and convenient detection (Figures 11 and 12, next page)
- Choice of a separate tube of ROX Reference Dye for optimization on any instrument, or ROX premixed for ultimate convenience on the ABI 7000, 7300, 7700, and 7900 instruments

Figure 9 — SuperScript™ III Platinum® One-Step qRT-PCR SuperMix with ROX shows better sensitivity and PCR efficiency than ABI's MasterMix



Panel A: Amplification plots comparing Invitrogen's SuperScript™ III Platinum® One-Step qRT-PCR SuperMix with ROX and ABI's TaqMan® One-Step RT-PCR MasterMix. HeLa total RNA in ten-fold serial dilutions from 100 ng to 1 pg was used as template, and reactions were run on an ABI PRISM® 7700 using ABI's recommended cycling protocol. Even with ABI's protocol, the Invitrogen reagents outperformed the ABI reagents by over 4 C_ts. **Panel B:** Standard curves generated from C_t values vs. relative template copy numbers of HeLa total RNA. Copy numbers are based on ten-fold serial dilutions of HeLa total RNA template. Amplifications were done using β-actin TaqMan® probes on an ABI PRISM® 7700 Instrument following ABI's recommended protocol. Even with ABI's protocol, the Invitrogen reagents outperformed the ABI reagents (.9993 vs. .9914).

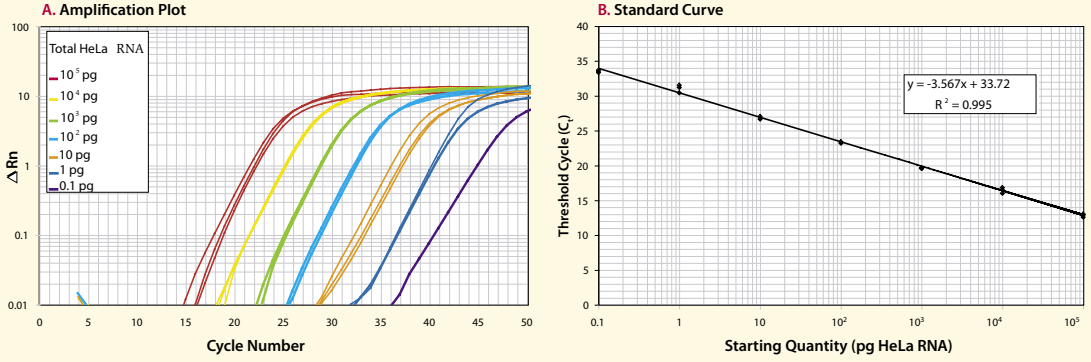
Figure 10 —Get greater sensitivity with the SuperScript™ III Platinum® One-Step qRT-PCR Kit



The human β-actin target was quantified by qRT-PCR using 10-fold serial dilutions of total HeLa RNA (100 ng to 0.1 pg) with either the SuperScript™ III Platinum® One-Step qRT-PCR Kit from Invitrogen (red) or the Quantitect™ Probe RT-PCR Kit from Qiagen (blue), 200 nM each amplification primer, 100 nM TaqMan® Probe, and ROX Reference Dye. cDNA synthesis was performed at 50°C for 15 min, followed by 40 cycles of PCR using an ABI PRISM® 7700.

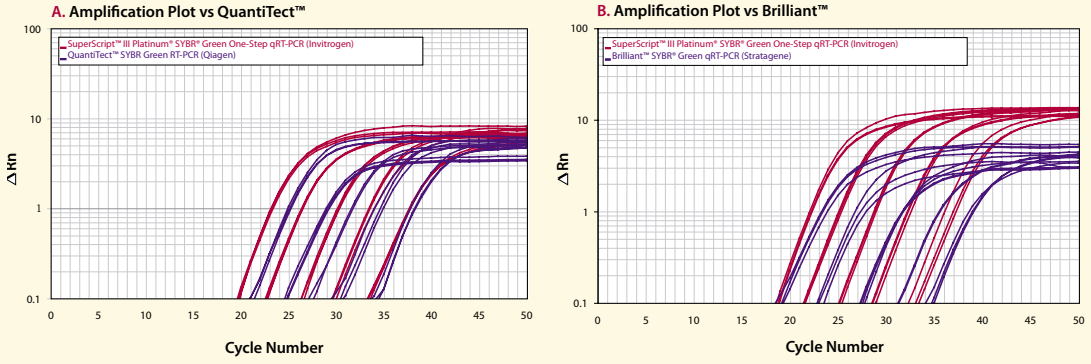
Sensitive one-step qRT-PCR with SuperScript™ III RT (cont.)

Figure 11— The SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit provides convenient detection across a broad dynamic range



qPCR of 10-fold serial dilutions (10^5 to 0.1 pg) of total HeLa RNA with 200 nM each GAPDH primer using the SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit. All reactions were performed on the ABI PRISM® 7000 using the following protocol: 3 min RT at 50ºC, followed by 50 cycles of 95ºC for 15 sec and 60ºC for 30 sec.

Figure 12— The SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit outperforms the competition



Panel A: qPCR of 10-fold serial dilutions (10 ng to 1 pg) of total HeLa RNA was performed with 200 nM each β-Actin primer using the SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit from Invitrogen (red lines; standard curve: y = -3.57x + 31.05; R^2 = 0.998) or the QuantiTect™ SYBR® Green RT-PCR Kit from Qiagen (blue lines; standard curve: y = -3.612x + 32.10; R^2 = 0.981) according to each manufacturer's protocol. Reactions were performed on the ABI PRISM® 7000. **Panel B:** qPCR of 10-fold serial dilutions (10 ng to 1 pg) of total HeLa RNA was performed with 200 nM each GAPDH primer using the SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit from Invitrogen (red lines; standard curve: y = -3.52x + 31.15; R^2 = 0.995) or the Brilliant® SYBR® Green qRT-PCR Kit from Stratagene (blue lines; standard curve: y = -3.893x + 32.89; R^2 = 0.996) according to each manufacturer's protocol. Reactions were performed on the ABI PRISM® 7000.

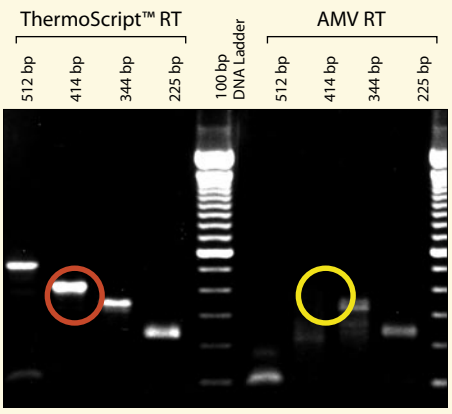
Product	Quantity	Rxn size	Cat. no.
For LUX™ primers or probe-based detection			
SuperScript™ III Platinum® One-Step qRT-PCR Kit	100 rxns	50 µl	11732-020
(for any instrument)	500 rxns	50 µl	11732-088
SuperScript™ III Platinum® One-Step qRT-PCR Kit with ROX	100 rxns	50 µl	11745-100
(for ABI instruments - 7000, 7300, 7700, 7900)	500 rxns	50 µl	11745-500
For SYBR® Green-based detection			
SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit	100 rxns	50 µl	11736-051
(for any instrument)	500 rxns	50 µl	11736-059
SuperScript™ III Platinum® with SYBR® Green One-Step qRT-PCR Kit with ROX	100 rxns	50 µl	11746-100
(for ABI instruments - 7000, 7300, 7700, 7900)	500 rxns	50 µl	11746-500

Sensitive one-step qRT-PCR with ThermoScript™ RT

Platinum® qRT-PCR ThermoScript™ One-Step System uses ThermoScript™ RT, a cloned avian reverse transcriptase to provide convenient and specific qRT-PCR results from total or poly(A)+ RNA in a single step. The Platinum® qRT-PCR ThermoScript™ One-Step System:

- Provides cDNA synthesis up to 70°C for better priming specificity and greater success with RNA secondary structure (Figure 13)
- Is optimized to provide maximum performance with LUX™ Fluorogenic Primers and dual-labeled fluorogenic probes (Figure 14)
- Reduces RNase H activity for greater first-strand cDNA yield
- Detects as few as 10 molecules of RNA template

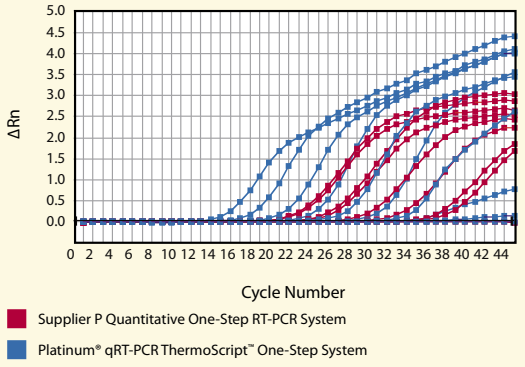
Figure 13— High-yield RT-PCR of GC-rich RNA with extensive secondary structure



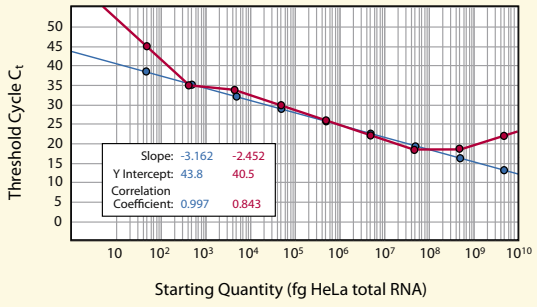
cDNA was synthesized from 10 ng total RNA using 15 units of ThermoScript™ RT or 15 units AMV RT according to manufacturers' recommendations. One tenth of each reaction was amplified with Platinum® *Taq* DNA Polymerase High Fidelity.

Figure 14 — High yield with Platinum® qRT-PCR ThermoScript™ One-Step System

A. Amplification Plot



B. Standard Curve



qRT-PCR of a 145-bp β-actin fragment was performed with the Platinum® qRT-PCR ThermoScript™ One-Step System (blue) or Supplier P Quantitative One-Step RT-PCR System (red) according to manufacturers' recommendations. Starting RNA varied from 5 µg to 50 fg in 10-fold serial dilutions. Normalized relative fluorescence (ΔRn) was collected using TAMRA as the passive reference. **Panel A:** Linear scale amplification plots. **Panel B:** Standard curve plots.

Product	Quantity	Rxn size	Cat. no.
Platinum® qRT-PCR ThermoScript™ One-Step System	100 rxns	50 µl	11731-015
(for any instrument)	500 rxns	50 µl	11731-023

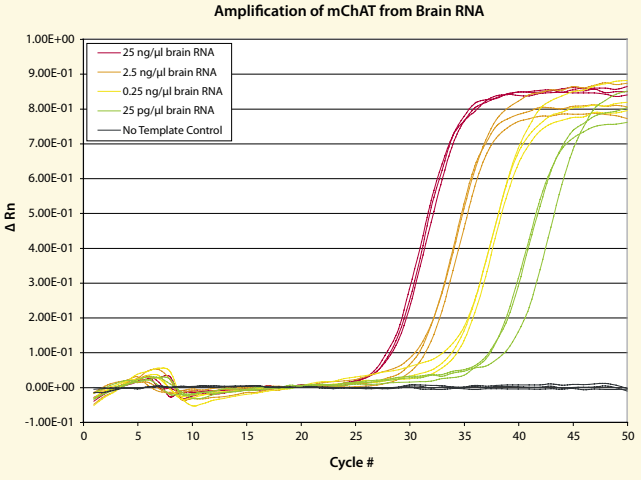
Ultra-sensitive qRT-PCR for RNA viruses and low abundance targets

The RNA UltraSense™ One-Step qRT-PCR System is specifically designed for amplification and real-time detection of RNA viruses and ultra-low abundance transcripts. The optimized, ultra-concentrated system combines SuperScript™ III Reverse Transcriptase (RT) and Platinum® *Taq* DNA Polymerase to provide the most sensitive and specific one-step system available for qRT-PCR. As a result, you achieve higher product yields and detection over a broad dynamic range.

Ultra-concentrated system for sensitive amplification of low-abundance RNA

The RNA UltraSense™ System is particularly useful for the study of rare transcripts or amplification of dilute templates. The 5X qRT-PCR reaction mix permits samples to be ~70% of the reaction mixture volume —2.5X more concentrated than with other qRT-PCR systems. This higher concentration formulation provides greater flexibility with low-concentration/high-volume RNA samples. As little as 25 pg/µl total RNA can be detected with a PCR efficiency of 97% (Figure 15).

Figure 15— High-sensitivity RNA detection and qRT-PCR efficiency with the RNA UltraSense™ One-Step qRT-PCR System



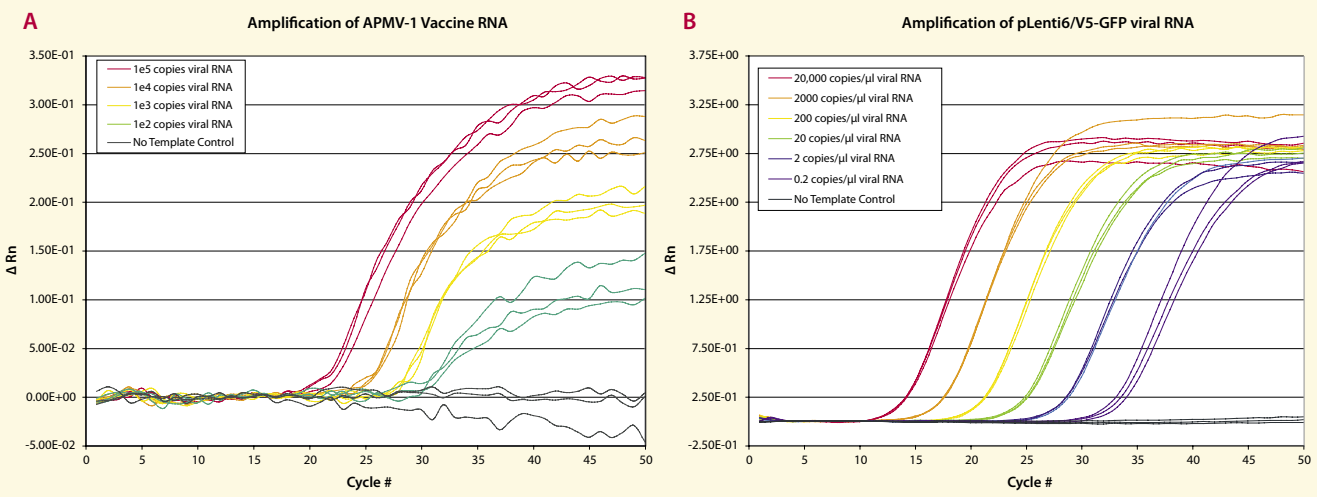
Total RNA from mouse brain was diluted to 25 ng/µl, 2.5 ng/µl, 0.25 ng/µl and 25 pg/µl. Thirteen microliters of each dilution were added to triplicate 20-µl reactions. The RNA UltraSense™ One-Step qRT-PCR System was able to amplify the rare ChAT message from RNA samples as dilute as 25 pg/µl with a PCR efficiency of 97%.

Ultra-sensitive qRT-PCR (cont.)

Unparalleled success with RNA secondary structure

RNA, especially viral RNA, can be extremely difficult to amplify due to the presence of significant secondary structure. The RNA UltraSense™ One-Step qRT-PCR System includes a proprietary enzyme mixture that improves performance by promoting primer-template interactions with difficult samples rich in secondary structure (Figure 16). In addition, SuperScript™ III RT enables cDNA synthesis at temperatures up to 60°C. This high thermostability increases success with targets rich in RNA secondary structure.

Figure 16— The RNA UltraSense™ qRT-PCR System provides improved amplification of viral RNA with significant secondary structure



The Newcastle virus is a single-stranded RNA virus that exhibits considerable secondary structure. qRT-PCR was performed on 10^5, 10^4, 10^3, 10^2, or 10^1 copies of END RNA using TaqMan® primers and probe against viral sequence. Immediately before loading, the templates were heated to 95ºC for 5 minutes and then placed on ice. **Panel A:** RNA UltraSense™ One-Step qRT-PCR System with SuperScript™ III RT provides clean amplification of four serial dilutions of RNA with exceptional efficiency (107%). A standard, competing one-step qRT-PCR system exhibits poor performance when encountering significant secondary structure. The standard system is only able to amplify two serial dilutions of RNA template. **Panel B:** Amplification of 6 serial dilutions of pLenti6/V5-GFP viral RNA with 90% efficiency.

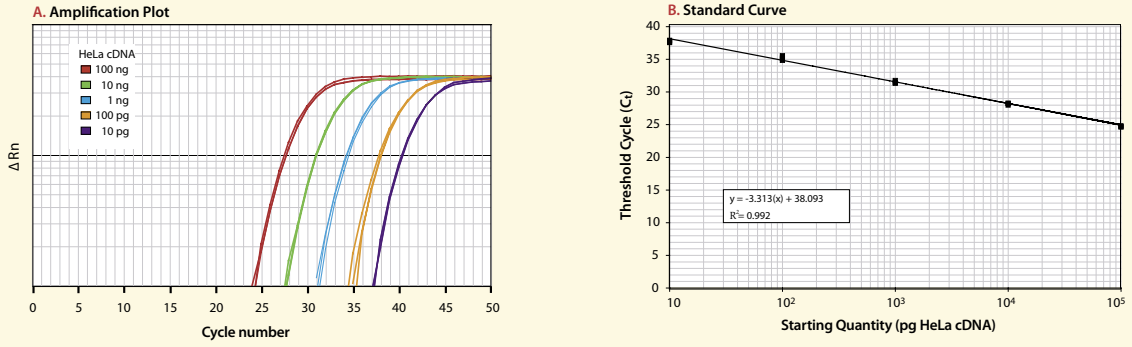
Product	Quantity	Rxn size	Cat. no.
RNA UltraSense™ One-Step qRT-PCR System (for any instrument)	100 rxns	50 µl	11732-927

Flexible two-step qRT-PCR

SuperScript™ III Platinum® Two-Step qRT-PCR Kits provide sensitive and specific qRT-PCR in a flexible two-step format. These kits are comprised of separate RT and PCR modules, giving you the ability to perform multiple PCR amplifications from a single cDNA reaction. These kits include Platinum® qPCR SuperMix-UDG for sensitive, specific detection with either LUX™ fluorogenic primers (Figure 17), dual-labeled probes (Figure 18), or SYBR® Green I dye (Figure 19, next page). All SuperScript™ III Platinum® Two-Step qRT-PCR Kits provide:

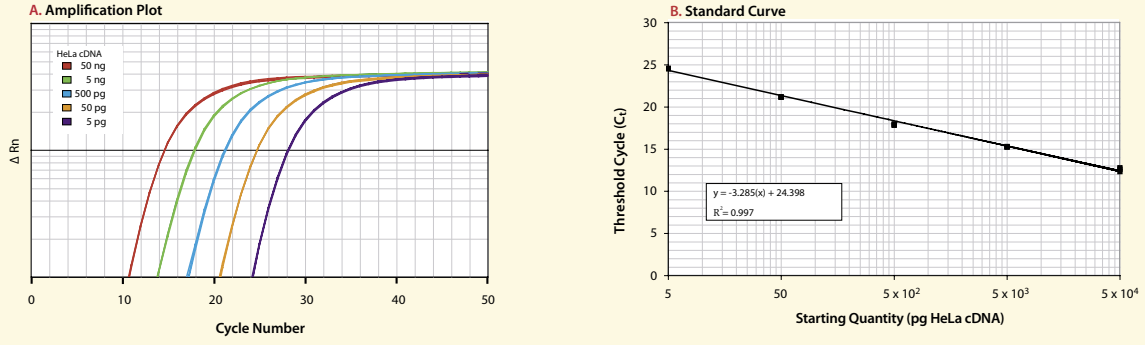
- SuperScript™ III Reverse Transcriptase for cDNA synthesis up to 60°C, giving you better success with difficult RNA secondary structure
- Platinum® *Taq* DNA Polymerase with hot-start technology for improved specificity
- Choice of a separate tube of ROX Reference Dye for optimization on any instrument, or ROX premixed for ultimate convenience on the ABI 7000, 7300, 7700, and 7900 instruments
- Sensitive quantitation from as little as 5 pg total RNA

Figure 17 — The SuperScript™ III Platinum® Two-Step qRT-PCR Kit provides specific detection with LUX™ Fluorogenic Primers



The human SDHA gene was quantified by qPCR using 10-fold serial dilutions (100 ng to 10 pg) of HeLa cDNA with 200 nM JOE-labeled LUX™ Primer, 200 nM unlabeled LUX™ Primer, SuperScript™ III Platinum® Two-Step qRT-PCR Kit, and ROX Reference Dye. Reactions were incubated for 2 min. at 50°C, then 2 min. at 95°C followed by 50 cycles of 95°C for 15 sec.; 60°C for 30 sec. using an ABI PRISM® 7700.

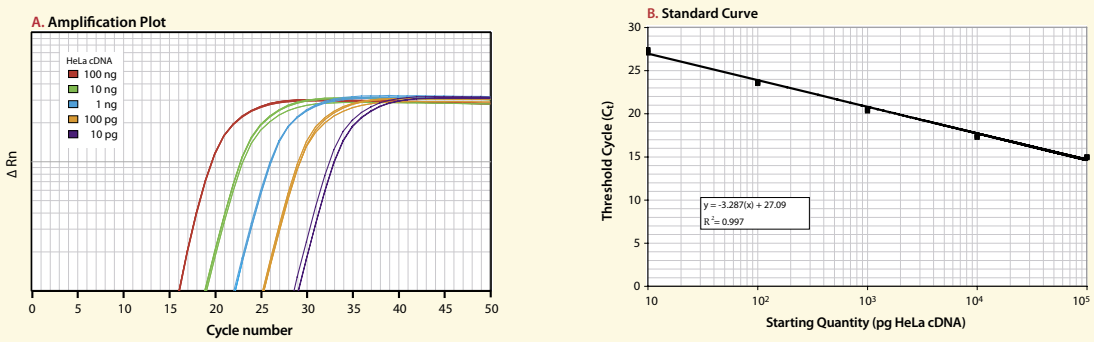
Figure 18 — The SuperScript™ III Platinum® Two-Step qRT-PCR Kit provides sensitive detection with TaqMan® Probes



Human 18S rRNA was quantified by qPCR using 10-fold serial dilutions (50 ng to 5 pg) of HeLa cDNA with 200 nM each amplification primer, 100 nM TaqMan® Probe, SuperScript™ III Platinum® Two-Step qRT-PCR Kit, and ROX Reference Dye. Reactions were incubated for 2 min. at 50°C, then 2 min. at 95°C followed by 50 cycles of 95°C for 15 sec.; 60°C for 30 sec. using an ABI PRISM® 7700.

Flexible two-step qRT-PCR (cont.)

Figure 19 — The SuperScript™ III Platinum® Two-Step qRT-PCR Kit with SYBR® Green provides easy and convenient detection with SYBR® Green I Dye



The human GAPDH gene was quantified by qPCR using 10-fold serial dilutions (100 ng to 10 pg) of HeLa cDNA with 200 nM each amplification primer, SuperScript™ III Platinum® Two-Step qRT-PCR Kit with SYBR® Green, and ROX Reference Dye. Reactions were incubated for 2 min. at 50˚C, then 2 min. at 95˚C followed by 50 cycles of 95˚C for 15 sec.; 60˚C for 30 sec. using an ABI PRISM® 7700.

Product	Quantity	Rxn size	Cat. no.
For LUX™ Primers or probe-based detection			
SuperScript™ III Platinum® Two-Step qRT-PCR Kit	100 rxns	50 µl	11734-050
(for any instrument)	500 rxns	50 µl	11734-068
SuperScript™ III Platinum® Two-Step qRT-PCR Kit with ROX	100 rxns	50 µl	11747-100
(for ABI instruments - 7000, 7300, 7700, 7900)	500 rxns	50 µl	11747-500
For SYBR® Green-based detection			
SuperScript™ III Platinum® SYBR® Green Two-Step qRT-PCR Kit	100 rxns	50 µl	11735-032
(for any instrument)	500 rxns	50 µl	11735-040
SuperScript™ III Platinum® SYBR® Green Two-Step qRT-PCR Kit with ROX	100 rxns	50 µl	11748-100
(for ABI instruments - 7000, 7300, 7700, 7900)	500 rxns	50 µl	11748-500

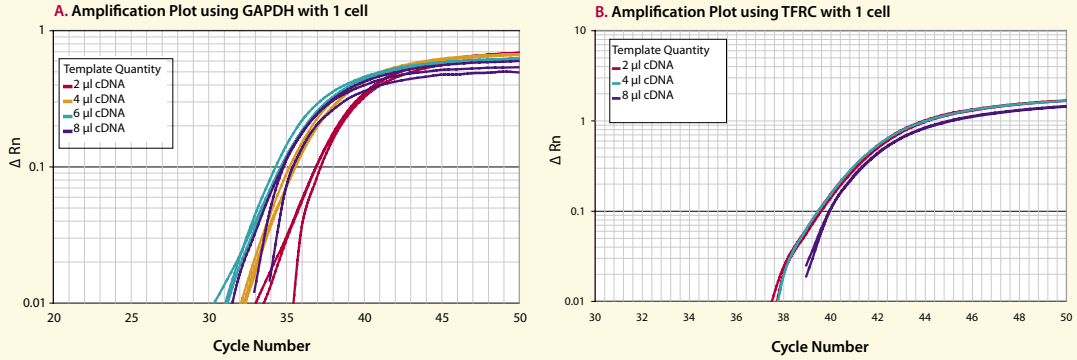
Simple qRT-PCR directly from cells

The SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit combines high yields of first-strand cDNA from SuperScript™ III RT with the hot-start specificity of Platinum® *Taq* DNA Polymerase to deliver sensitive, specific, qPCR results directly from cells. Both kits provide:

- High-yield cDNA synthesis and qPCR detection from a single cell up to 10,000 cells (Figure 20)
- Single-tube format without a separate RNA purification step eliminates sample loss and enables detection of rare transcripts
- A simple, convenient protocol that produces superior results across a range of sample quantities (Figure 21)

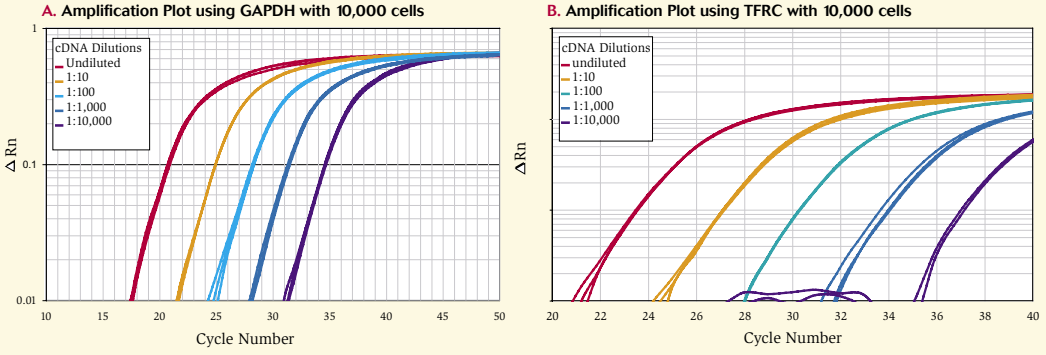
SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit includes Platinum® qPCR SuperMix-UDG for superior detection with LUX™ Fluorogenic Primers (Figure 23A, next page), dual-labeled fluorogenic probes (Figure 22, next page), or SYBR Green I dye (Figure 23B, next page)

Figure 20 — Single-cell detection with the SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit



qPCR was performed on 2, 4, 6 (GAPDH only), and 8 µl of cDNA synthesized from a single HeLa cell (by dilution) using the SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit with 200 nM each primer and 100 nM TaqMan® Probe specific to either GAPDH (Panel A) or TFRC (Panel B) housekeeping genes. Fifty-microliter reactions were incubated for 2 min. at 50°C, then 2 min. at 95°C, followed by 50 cycles of 95°C for 15 sec.; 60°C for 30 sec. using the ABI PRISM® 7700.

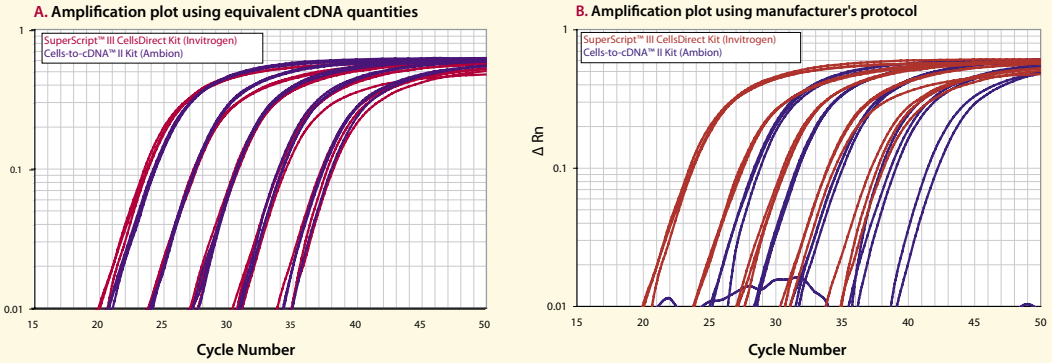
Figure 21 — SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit detects up to 10,000 cells



qPCR was performed on 10-fold serial dilutions (undiluted to 1:10,000) of cDNA synthesized from 10,000 HeLa cells using the SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit. Four microliters of each dilution were added to a 50-µl qPCR reaction containing 200 nM each primer and 100 nM TaqMan® Probe to GAPDH (Panel A; standard curve: $y = -3.37x + 36.315$; $R^2 = 0.9967$) or TFRC (Panel B; standard curve: $y = -3.395(x) + 41.5$; $R^2 = 0.999$) housekeeping genes. Reactions were incubated for 2 min. at 50°C, then 2 min. at 95°C followed by 50 cycles of 95°C for 15 sec.; 60°C for 30 sec. using the ABI PRISM® 7700.

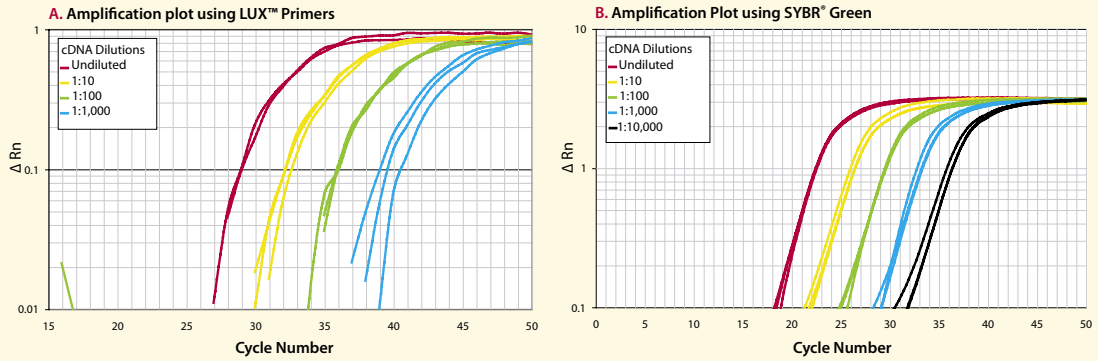
Simple qRT-PCR directly from cells (cont.)

Figure 22—SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit outperforms the competition



qPCR was performed on 10-fold serial dilutions of cDNA synthesized from HeLa cells using the SuperScript™ III Platinum® CellsDirect Two-Step qRT-PCR Kit from Invitrogen (red) or the Cells-to-cDNA™ II Kit from Ambion (blue). Four microliters of each dilution were added to a 50-µl qPCR reaction containing 200 nM each TaqMan® primer and 100 nM probe specific to GAPDH and amplified with a standard protocol on the ABI PRISM® 7700. **Panel A:** Results comparing cDNA synthesized from 1,000 cells with the SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit (standard curve: y = 3.48x + 39.376; R^2 = 0.9968) or 10,000 cells with the Cells-to-cDNA™ II Kit (standard curve: y = -3.33x + 39.047: R^2 = 0.9982). Because of protocol differences, the Cells-to-cDNA™ II Kit requires 10X more starting sample to produce the cDNA equivalent of 1,000 cells. **Panel B:** Results comparing cDNA synthesized from 1,000 cells with the SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit (standard curve: y = -3.48x + 39.376; R^2 = 0.9968) and 1,000 cells with the Cells-to-cDNA™ II Kit (standard curve: y = -3.35x + 43.367: R^2 = 0.995) following each manufacturer's suggested protocol.

Figure 23—The SuperScript™ III Platinum® CellsDirect Two-Step qRT-PCR Kit provides sensitive detection with LUX™ Primers or SYBR® Green I



qPCR was performed on 10-fold serial dilutions of cDNA synthesized from 1000 HeLa cells using the SuperScript™ III Platinum® CellsDirect Two-Step qRT-PCR Kit. Four microliters of each dilution were added to a 50-µl qPCR reaction with ROX Reference Dye and the reagents indicated. Reactions were incubated for 2 min. at 50°C, then 2 min. at 95°C, followed by 50 cycles of 95°C for 15 sec.; 60°C for 30 sec. using the ABI PRISM® 7700. **Panel A:** Amplification and detection using 200 nM each LUX™ Primer specific to the SDHA housekeeping gene. Standard curve: y = -3.539x + 42.808; R^2 = 0.99 **Panel B:** Amplification and detection with SYBR® Green using 200 nM each primer specific to the β-actin housekeeping gene. Standard curve: y = -3.375x + 29.348; R^2 = 0.995

Product	Quantity	Rxn size	Cat. no.
For LUX™ Primers-based and probe-based detection			
SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit	100 rxns	50 µl	11737-030
(for any instrument)	500 rxns	50 µl	11737-038
For SYBR® Green-based detection			
SuperScript™ III Platinum® CellsDirect™ Two-Step qRT-PCR Kit with SYBR® Green	100 rxns	50 µl	11738-060
(for any instrument)	500 rxns	50 µl	11738-068
CellsDirect™ Resuspension and Lysis Buffer (includes 10 ml resuspension buffer and 1 ml lysis buffer)	1 Kit		11739-010

Convenient Room Temperature Stable (RTS) qPCR SuperMixes

Convenient qPCR SuperMixes

Platinum® RTS qPCR SuperMixes provide high-performance qPCR from convenient lyophilized reagents. Dry, individual aliquots require no thawing and speed reaction set-up (Figure 24), while maintaining sensitive, specific amplification and detection. Platinum® RTS qPCR SuperMixes offer:

- Faster setup—no thawing or aliquotting of reagents needed
- Greater convenience—single-use aliquots minimize pipetting, saving time and reducing contamination risk
- Research flexibility with room temperature shipping and storage
- Higher specificity from Platinum® *Taq* DNA Polymerase
- Compatibility with multiple detection formats, including LUX™ Primers (Figure 25A), dual-labeled fluorogenic probes (Figure 25B), and SYBR® Green I Dye (Figure 26, next page)
- Compatibility with a wide range of instrument systems

Figure 24— RTS Amplification Reagents speed reaction setup

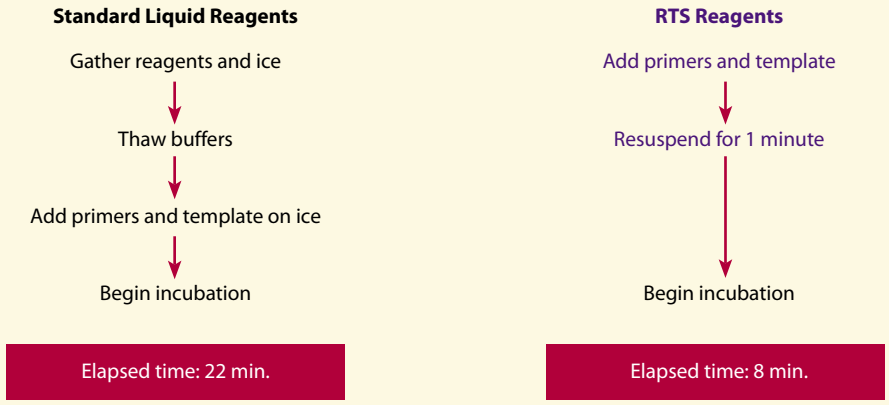
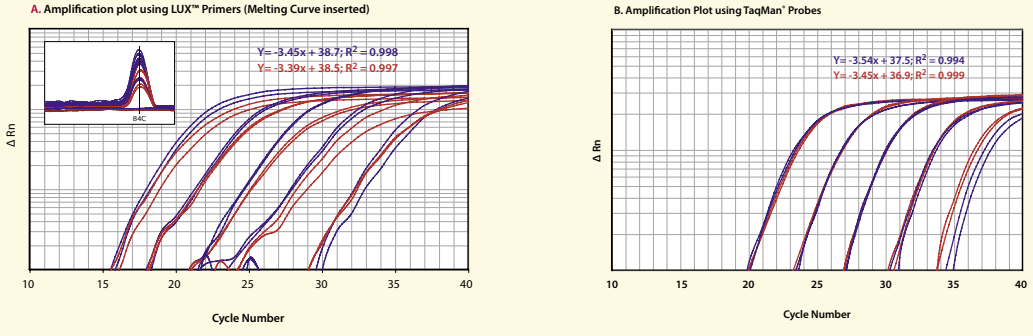


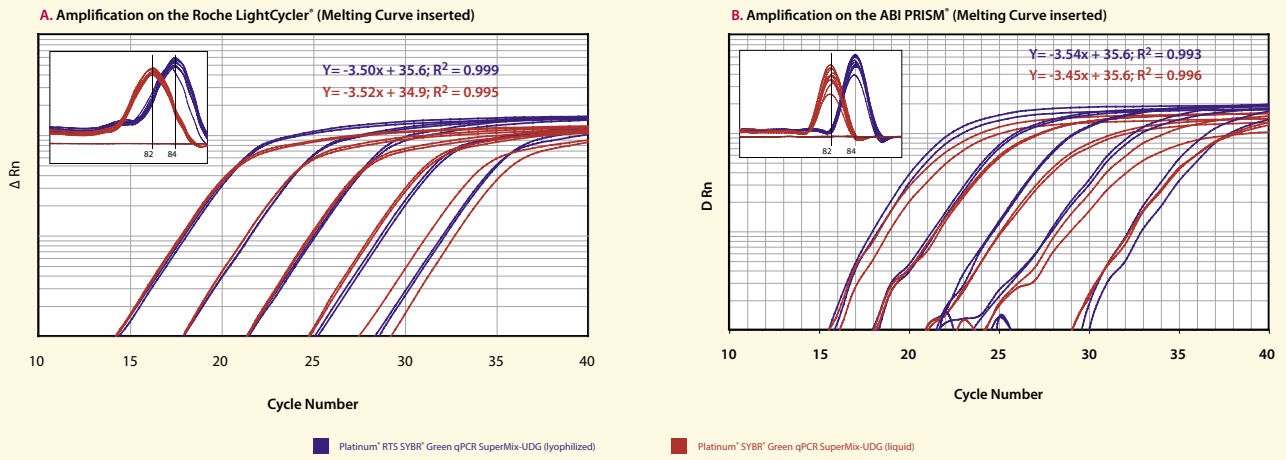
Figure 25— Platinum® RTS qPCR SuperMix-UDG (blue) provides similar performance to liquid Platinum® qPCR SuperMix-UDG (red) with LUX™ Fluorogenic Primers and TaqMan® Probes



qPCR of 10-fold serial dilutions (100 ng-10 pg) of HeLa cDNA was performed using 200 nM each LUX® Primers (Panel A) or 200 nM each primer and 100 nM TaqMan® Probe (Panel B) specific to the β-actin housekeeping gene with Platinum® qPCR SuperMix-UDG (red) or with Platinum® RTS qPCR SuperMix-UDG (blue) and ROX Reference Dye. Reactions were incubated for 2 min. at 50˚C, then 2 min. at 95˚C, followed by 40 cycles of 95˚C for 15 sec.; 60˚C for 30 sec. using the ABI PRISM® 7700.

Convenient Room Temperature Stable (RTS) qPCR SuperMixes (cont.)

Figure 26— Platinum® RTS SYBR® Green qPCR SuperMix-UDG (blue) provides similar performance to liquid Platinum® SYBR® Green qPCR SuperMix-UDG (red) on multiple instrument platforms.



Panel A: qPCR of 10-fold serial dilutions (100 ng-10 pg) of HeLa cDNA was performed using primers (200 nM each) specific to the GAPDH housekeeping gene with Platinum® SYBR® Green qPCR SuperMix-UDG and additional BSA (red) or with Platinum® RTS SYBR® Green qPCR SuperMix-UDG (blue). Reactions were incubated for 1 min. at 50˚C, then 1 min. at 92˚C, followed by 40 cycles of 95˚C for 5 sec.; 60˚C for 30 sec. using the Roche LightCycler®. **Panel B:** qPCR of 10-fold serial dilutions (100 ng-10 pg) of HeLa cDNA was performed using primers (200 nM each) specific to the GAPDH housekeeping gene with Platinum® SYBR® Green qPCR SuperMix-UDG (red) and or with Platinum® RTS SYBR® Green qPCR SuperMix-UDG (blue) and ROX Reference Dye. Reactions were incubated for 2 min. at 50˚C, then 2 min. at 95˚C, followed by 40 cycles of 95˚C for 15 sec.; 60˚C for 30 sec. using the ABI PRISM® 7000.

Product	Quantity	Rxn size	Cat. no.
For LUX™ Primers or probe-based detection			
Platinum® RTS qPCR SuperMix-UDG	5 x 96-well plates	25 µl	11730-033
(for any instrument)	12 x 8 strip tubes	25 µl	11730-058
Platinum® RTS qPCR SuperMix-UDG with ROX (for ABI instruments - 7000, 7300, 7700, 7900)	5 x 96-well plates	25 µl	11730-041
For SYBR® Green-based detection			
Platinum® RTS SYBR® Green qPCR SuperMix-UDG	5 x 96-well plates	25 µl	11733-052
(for any instrument)	12 x 8 strip tubes	25 µl	11733-078
Platinum® RTS SYBR® Green qPCR SuperMix-UDG 96 with ROX (for ABI instruments - 7000, 7300, 7700, 7900)	5 x 96-well plates	25 µl	11733-060

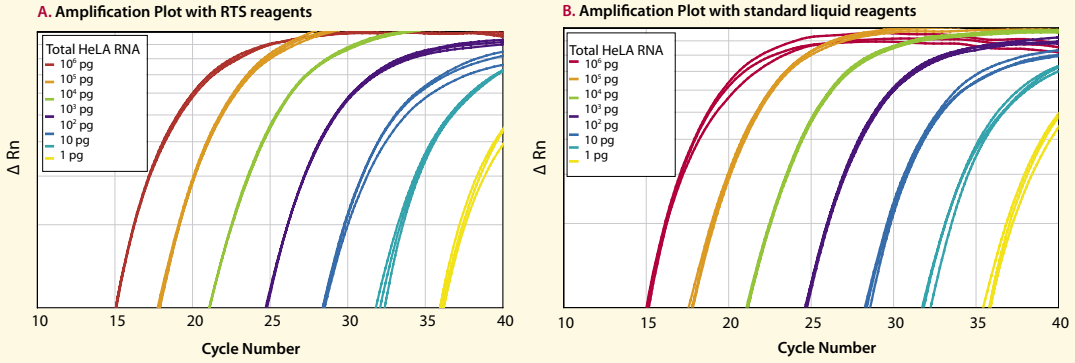
Convenient Room Temperature Stable (RTS) qRT-PCR SuperMixes

Sensitive one-step qRT-PCR

SuperScript™ III Platinum® RTS One-Step qRT-PCR Kits provide the same high-quality qRT-PCR results as liquid formulations (Figure 27), with faster reaction set-up, convenient transportation, and long-term storage at ambient temperature. SuperScript™ III Platinum® RTS One-Step qRT-PCR Kits offer:

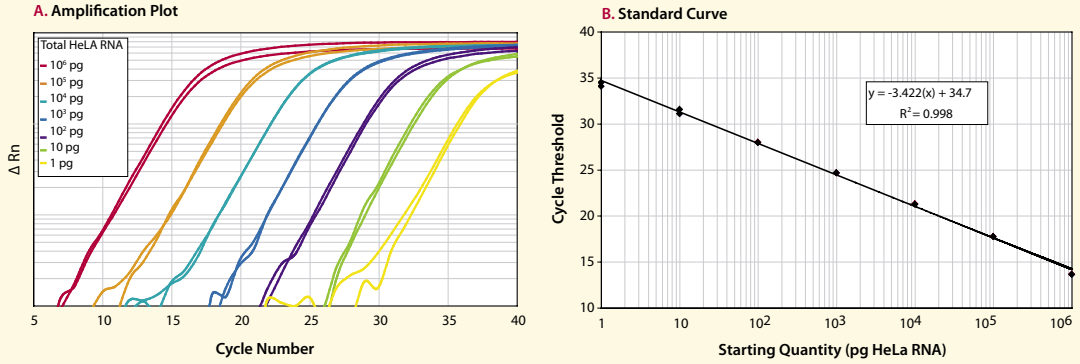
- SuperScript™ III RT for higher temperature cDNA synthesis for greater success with difficult RNA secondary structure
- Platinum® *Taq* DNA Polymerase with hot-start technology for improved specificity
- Superior performance with LUX™ Fluorogenic Primers or dual-labeled fluorogenic probes
- Flexible configurations with or without ROX Reference Dye
- Compatibility with multiple real-time instrument platforms, including rotor systems (Figure 28)

Figure 27— SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit provides similar performance to standard liquid SuperScript™ III Platinum® One-Step qRT-PCR Kit.



qPCR of 10-fold serial dilutions (10^6 to 1 pg) of total HeLa RNA was performed with TaqMan® primers and probe for GAPDH labeled with JOE-BHQ using the SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit with lyophilized reagents (A) or the standard liquid SuperScript™ III Platinum® One-Step qRT-PCR Kit (B). All reactions were performed on the ABI PRISM® 7000.

Figure 28— SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit provides sensitive amplification on the Roche LightCycler®



qPCR of 10-fold serial dilutions (106 to 1 pg) of total HeLa RNA was performed with TaqMan® primers and probe for β-Actin labeled with FAM-TAMRA using the SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit on the Roche LightCycler®.

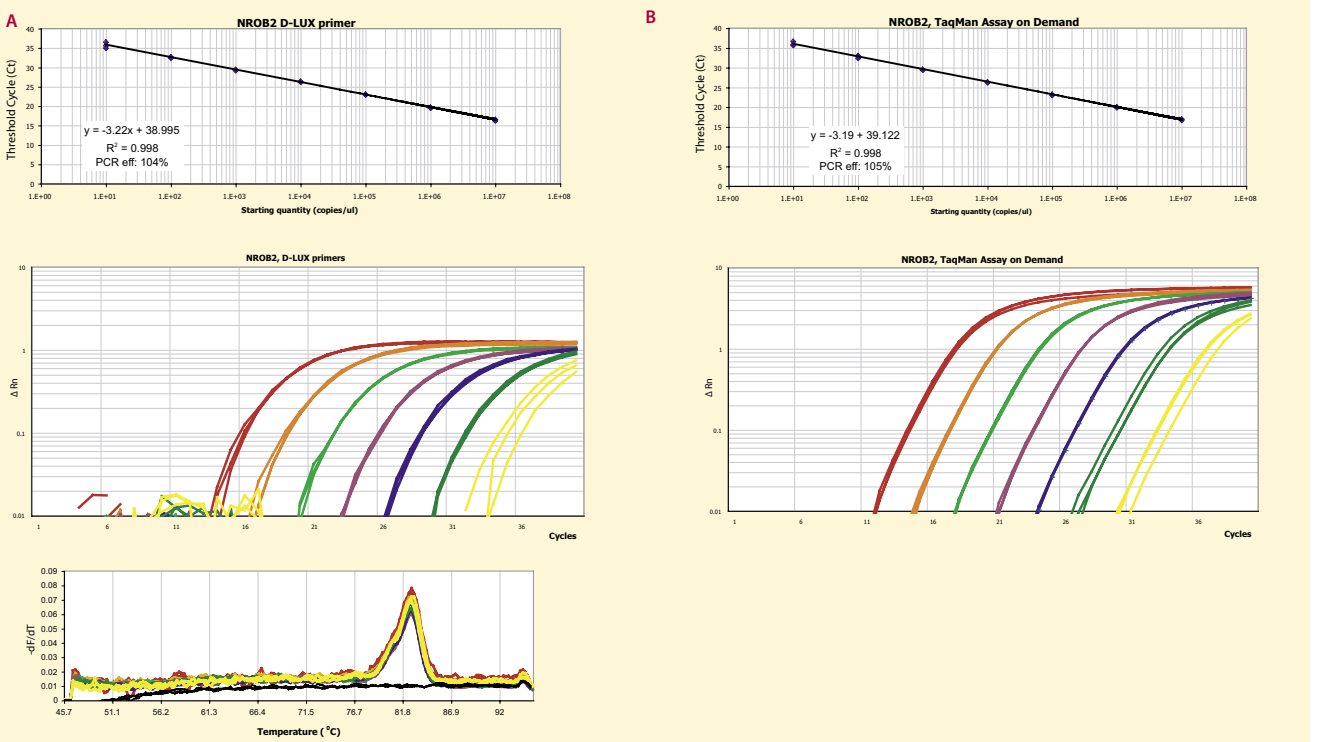
Product	Quantity	Rxn size	Cat. no.
SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit (for any instrument)	1 x 96-well plate	25 µl	11728-081
	12 x 8 strip wells	25 µl	11728-097
SuperScript™ III Platinum® RTS One-Step qRT-PCR Kit with ROX (for ABI instruments - 7000, 7300, 7700, 7900)	1 x 96-well plate	25 µl	11728-089

D-LUX™ Detection Platform for qPCR

The D-LUX™ Detection Platform combines improvements to our original LUX™ (Light Upon eXtension) detection technology with optimized bioinformatics to provide the most sensitive, specific, and cost-effective detection method available for qPCR/qRT-PCR. The D-LUX™ Detection Platform offers improved detection chemistry with specificity and sensitivity equal to TaqMan® probes (Figure 29), improved signal/background ratio, and an expanded array of software design features and functions for fast and easy primer design and higher performance primers. With the D-LUX™ Detection Platform, you can:

- Quantitate 10 or fewer copies with a dynamic range of up to eight orders of magnitude
- Perform melting curve analysis to distinguish genuine amplicons from primer-dimers and other artifacts
- Increase multiplexing capabilities with five fluorescent dye labels
- Perform quantitation on multiple real-time instrument platforms
- Design primers with confidence with our 100% performance guarantee

Figure 29 —D-LUX™ Detection Platform vs. TaqMan® Probes



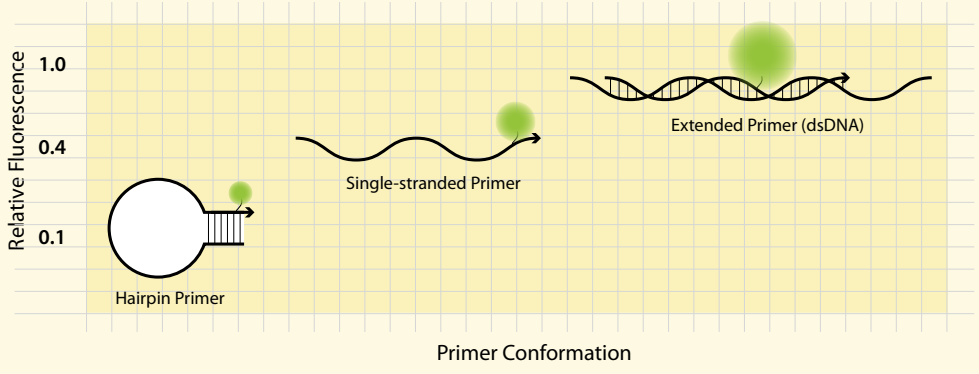
Side-by-side comparison of qPCR with LUX™ Primers designed using D-LUX™ Designer (A) and TaqMan® probe detection system using ABI's Gene Expression Assay (B) for human Human NROB2 (NM_021969) . Amplification was performed using Platinum® qPCR SuperMix-UDG using primer and probe amounts recommended by manufacturers. Human HMBS ORF clone was used with serial 10-fold dilution starting at 10^7 copies per reaction. Reactions were incubated for 2 min at 50°C, 2 min at 95°C, and 50 cycles of 15 sec 95°C, 30 sec at 55°C, and 30 sec at 72°C, followed by a melting curve using an ABI PRISM® 7700 instrument.

D-LUX™ Detection Platform for qPCR (cont.)

How LUX™ detection technology works

LUX™ Fluorogenic Primers Sets consist of one primer labeled with a single fluorophore and a corresponding unlabeled primer, both custom-synthesized according to the DNA/RNA of interest. Typically 20-30 bases in length, LUX™ Primers are designed with a fluorophore close to the 3´end in a hairpin structure. This configuration intrinsically renders fluorescence quenching capability, making a separate quenching moiety unnecessary. When the primer becomes incorporated into the double-stranded PCR product, the fluorophore is de-quenched, resulting in a significant increase in fluorescent signal (Figure 30). This signal increase is the basis for LUX™ detection.

Figure 30—The LUX™ (Light Upon eXtension) effect.



Ideal alternative to probe technology

The D-LUX™ platform provides the ideal alternative to probe technology. With TaqMan® Probes or Molecular Beacons, you need a pair of PCR primers in addition to a dual-labeled probe that hybridizes to the internal portion of the amplicon. Using the D-LUX™ platform, all you need is one fluorogenic primer labeled with a single reporter dye, and one corresponding unlabeled primer. The fluorogenic primer can be either forward or reverse. The result is simple primer design with fast and inexpensive production, allowing you to analyze more genes at a lower cost compared to TaqMan® Probes.

Discover the power of the D-LUX™ Detection Platform

Compared to dual-labeled probes or DNA binding dyes, LUX™ Fluorogenic Primers present an attractive alternative for qPCR detection. The D-LUX™ Detection Platform combines superior performance with a significant cost savings (Table 3).

Table 3—Comparison of real-time detection platforms

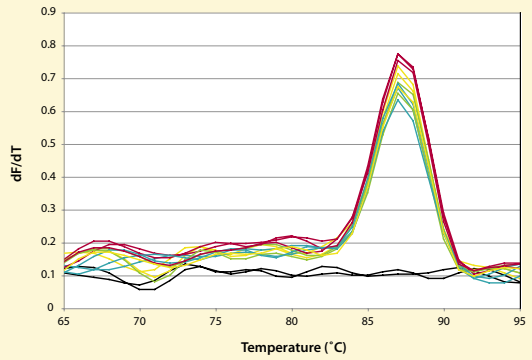
	TaqMan® Probes	Molecular Beacons	SYBR® Green I	LUX™ Primers
Sensitivity	• • •	• • •	•	• • •
Dynamic range	• • •	• • •	•	• • •
Specificity	• • •	• • •	•	• • •
Multiplexing	• •	• •	N/A	• • •
Melting curve analysis	N/A	N/A	• • •	• • •
Ease of design	•	•	• • •	• • •
Cost	•	•	• • •	• • •

D-LUX™ Detection Platform for qPCR (cont.)

Melting curve analysis

Melting curve analysis is a simple, straightforward way to check qPCR reactions for primer-dimer artifacts and contamination and to ensure reaction specificity and accurate quantification. LUX™ Fluorogenic Primers provide the flexibility you need to perform melting curve analysis on your qPCR samples. You can easily confirm the absence of primer-dimer artifacts or contaminants, giving you more information about your samples and greater confidence in your results. Figure 31 demonstrates a set of melting curves produced using LUX™ Fluorogenic Primers.

Figure 31—Melting curve analysis confirming specific amplification with LUX™ Primers



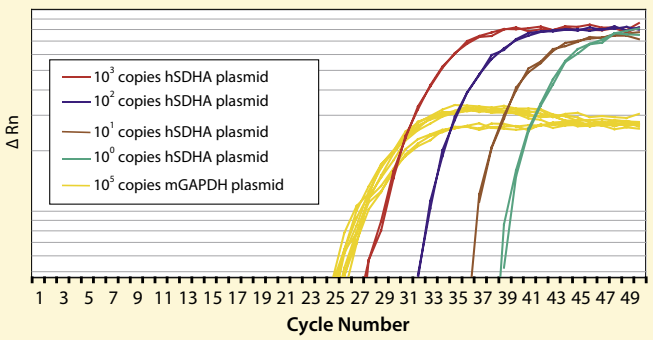
qPCR was performed on 10-fold serial dilutions (10^6 to 10^3 copies) of human β-actin plasmid using 200 nM FAM-labeled LUX™ Primer, 200 nM unlabeled primer, and Platinum® qPCR SuperMix-UDG. Reactions were amplified by 50 cycles of PCR using a Corbett Research Rotor-Gene™. Melting curve analysis was performed after amplification using a temperature ramp of 1˚C/5 sec. between 65˚C and 95˚C. Colored lines indicate template-specific reactions. Black lines indicate no-template controls.

Multiplex qPCR made easy with LUX™ Fluorogenic Primers

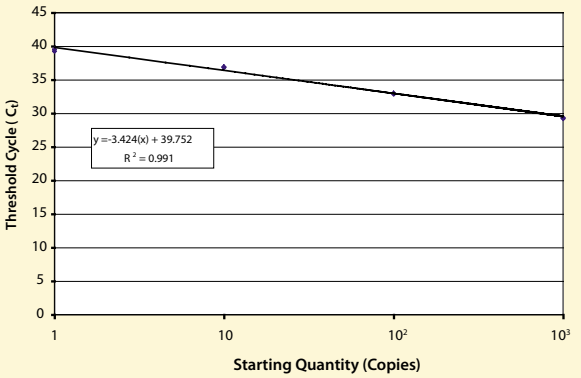
Multiplexing enables you to profile multiple genes in a single sample, saving you time and money. With LUX™ Primers, multiplexing is simple and efficient (Figure 32). All you need to do is design your primers using our D-LUX™ Designer Software and follow our simple multiplexing protocol (found at www.invitrogen.com/lux). With dual-labeled probe/primer systems you need to design and optimize two primers and a probe for each target, a process which is very difficult and time consuming. Binding dyes like SYBR® Green I lack any multiplexing capability. LUX™ Primers are available with FAM, JOE, HEX, TET, or Alexa Fluor® 546 dye labels.

Figure 32—Multiplexing is simple and efficient with LUX™ Primers

A. **(C_t) Amplification Plot**



B. **Standard Curve**



Multiplex qPCR with LUX™ Primers was performed using Platinum® qPCR SuperMix-UDG supplemented to a final concentration of 6 mM $MgCl_2$. 200 nM FAM-labeled LUX™ SDHA Primers were used for amplification of serial dilution of human cDNA and 100 nM JOE-labeled LUX™ GAPDH Primers were used for amplification of GAPDH added to the multiplex assays at a constant amount of 10^5 copies per assay. Reactions were incubated for 2 min at 50˚C, 2 min at 95˚C and 50 cycles of 15 sec 95˚C, 30 sec 55˚C, 30 sec 72˚C using an ABI PRISM® 7700.

D-LUX™ Designer Software

Simple primer design with D-LUX™ Designer software

The D-LUX™ Designer is the most comprehensive tool available for designing and ordering LUX™ Primers. It offers extensive features and functions to help you design the best primers for your gene of interest, including the identification of exon-exon junctions, automatic determination of intron size, and identification and alignment of potential splice variants (Figure 33). In addition, the D-LUX™ Designer offers:

- New algorithm for higher PCR primer performance
- Improved primer scoring system
- Guided step-by-step primer design
- Double BLAST analysis of your sequences (online version only)

Simply enter your target sequence and the software will generate primer sets ranked in order of optimization. Design and order custom LUX™ Primers online at www.invitrogen.com/dluxdesigner. To download the D-LUX™ Designer software or request a CD version of the software, please visit www.invitrogen.com/lux. Primers designed through the D-LUX™ Designer are available with the FAM, JOE, HET, TET, and ALEXA Fluor® 546 dye labels.

Figure 33—D-LUX™ Designer homepage

Product	Quantity
LUX™ Fluorogenic Primer Set, FAM-labeled	50 nmol
	200 nmol
LUX™ Fluorogenic Primer Set, JOE-labeled	50 nmol
	200 nmol
LUX™ Fluorogenic Primer Set, HEX-labeled	50 nmol
	200 nmol
LUX™ Fluorogenic Primer Set, TET-labeled	50 nmol
	200 nmol
LUX™ Fluorogenic Primer Set, Alexa Fluor® 546-labeled	50 nmol
	200 nmol

qPCR Instrument Calibration Kit for accurate qPCR results

The qPCR Instrument Calibration Kit includes qualified reagents for calibrating your real-time PCR system, providing you with data you can trust. This calibration will prepare your instrument to provide accurate, reliable results using LUX™ Fluorogenic Primers labeled with FAM, JOE, HEX, TET, and/or Alexa Fluor® 546 dyes.

The qPCR Instrument Calibration Kit is for use with real-time PCR instruments that have the option of being calibrated manually. The kit is compatible with, but not limited to, the ABI PRISM® 7000, ABI PRISM® 7700, ABI PRISM® 7900HT, and ABI 7500 Real-Time PCR System.

Product	Quantity	Cat. no.
qPCR Instrument Calibration Kit	1 kit	11742-100

Fast and easy detection with Certified LUX™ Primer Sets

Certified LUX™ Primer Sets for Human Genes

Ready-to-use LUX™ Primer Sets for reliable qPCR

Certified LUX™ Primer Sets for Human Genes are pre-made primer sets functionally validated and optimized for accurate quantification of your gene of interest. They are available with FAM labels and can be multiplexed with Certified LUX™ Primer Sets for Housekeeping Genes with JOE labels. These sets are ideal for validating microarray data or for use with Stealth™ RNAi experiments. Invitrogen now offers more than 300 Certified LUX™ Primer Sets for Human Genes.

Certified LUX™ Primer Sets for Housekeeping Genes

Accurate real-time normalization

Certified LUX™ Primer Sets for Housekeeping Genes are designed for use in conjunction with custom-designed, target-specific LUX™ Fluorogenic Primers to provide reliable normalization for qPCR. Each premade primer set has been functionally validated and optimized for accurate quantification of a corresponding stable control gene with reliable normalization data. The wide range of genes, species, and expression levels represented enables you to match the expression characteristics of your target gene, for greater normalization accuracy. Certified LUX™ Primer Sets for Housekeeping Genes:

- Offer sensitive, specific, cost-effective normalization in a ready-to-use format
- Are functionally validated for human, mouse/rat, and *Drosophila* control genes
- Represent multiple expression levels to match your target expression level
- Are available with either FAM or JOE labels

For a complete list of available primer sets please visit us at **www.invitrogen.com/lux** or use our database search at **www.invitrogen.com/certifiedluxsearch**.

Product	Quantity	Rxn size	Cat. no.
Certified LUX™ Primer Sets for Human Genes, FAM labeled	100 rxn	50 µl	www.invitrogen.com/lux
Certified LUX™ Primer Sets for Housekeeping Genes, FAM labeled	100 rxn	50 µl	www.invitrogen.com/lux
Certified LUX™ Primer Sets for Housekeeping Genes, JOE-labeled	100 rxn	50 µl	www.invitrogen.com/lux

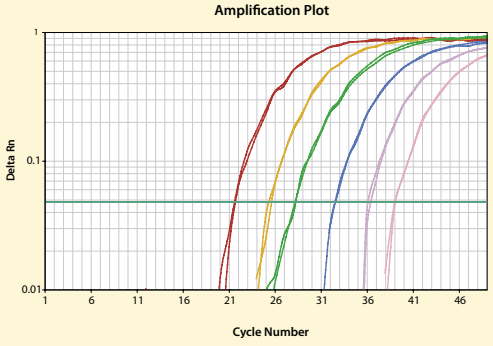
Certified LUX™ Primer Sets for infectious agents

LUX™ Technology provides specific, sensitive qPCR pathogen analysis

Certified LUX™ Primer Sets for Infectious Agents provide highly specific, sensitive detection and quantification of pathogen domains by qPCR (Figure 34). Each Certified LUX™ Primer Set for Infectious Agents has been designed and functionally validated to amplify characteristic genetic regions to specifically identify pathogenic organisms. Certified LUX™ Primer Sets for Infectious Agents provide:

- Specific, sensitive real-time pathogen detection and/or quantification
- Post-amplification analysis capability to confirm accurate sequence amplification
- Cost-effective amplification in a ready-to-use format

Figure 34 —Amplification plot of Certified LUX™ Primer Set for SARS Coronavirus

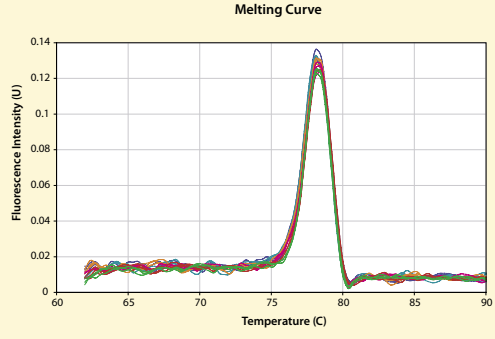


Serial dilution of cDNA from total SARS Coronavirus RNA were amplified using Platinum® qPCR SuperMix-UDG in 20 ml volumes with 200 nM FAM-labeled LUX™ primer, 200 nM reverse primer and ROX Reference Dye. PCR reactions were incubated 2 min at 50˚C and 2 min at 95˚C then cycles of: 95˚C. 15s; 60˚C. 30s on an ABI PRISM® 7700 sequence detection system. The highest concentration corresponds to 5 ng total RNA.

Post-amplification analysis capability to confirm accurate sequence amplification

Unlike other real-time detection technologies, LUX™ Fluorogenic Primers enable post-amplification analysis to confirm amplification of infectious agents. A rapid postamplification ramping protocol provides melting curve data between two temperature points. Within minutes, the melting curve output provides visual analysis of the PCR product to verify correct sequence amplification and eliminate false-negative results from nonspecific amplification (Figure 35).

Figure 35 —Melting curve analysis to verify nonspecific amplification of Certified LUX™ Primer Set for Infectious Agents



Serial dilutions of cDNA from total SARS Coronavirus RNA were amplified using Platinum® qPCR Super Mix-UDG in 20 ml volumes with 200 nM FAM-labeled LUX™ primer, 200 nM reverse primer and ROX Reference Dye. PCR reactions were incubated 2 min at 50˚C and 2 min at 95˚C then cycled for 50 cylces of: 95˚C. 15s; 60˚C. 30s on an ABI PRISM® 7700 sequence detection system.

For a complete list of available primer sets please visit us at **www.invitrogen.com/lux** or use our database search at **www.invitrogen.com/certifiedluxsearch**.

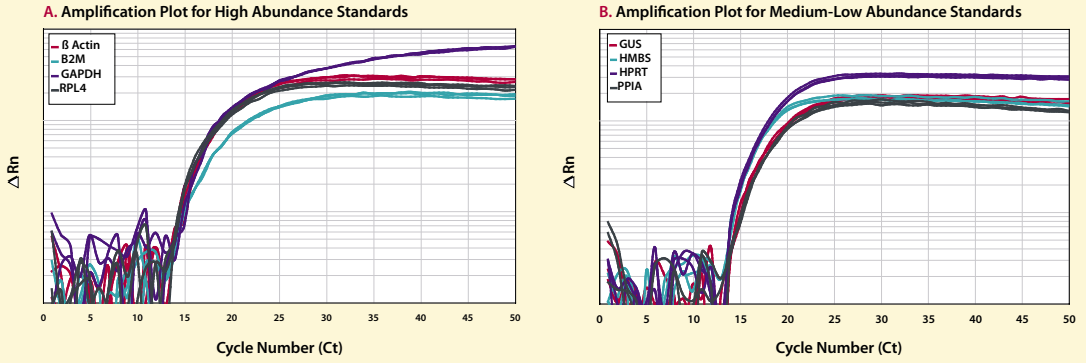
Product	Quantity	Rxn size	Cat. no.
Certified LUX™ Primer Sets for Infectious Agents, FAM labeled	250 rxn	20 µl	visit www.invitrogen.com/lux

Reliable qPCR standards

Invitrogen's qPCR Plasmid Standards provide a simple, reproducible method of determining gene copy numbers in qPCR using an external standard. qPCR Plasmid Standards are available for both high abundance and medium-low abundance housekeeping genes. Each formulation contains DNA qPCR standards for four housekeeping genes, supplied at 1 x 10^8 copies/µl. qPCR Plasmid Standards, High Abundance include β-Actin, B2M, GAPDH, and RPL4 (Figure 36A). qPCR Plasmid Standards, Medium-Low Abundance include GUS, HMBS, HPRT, and PPIA (Figure 36B). qPCR Plasmid Standards offer:

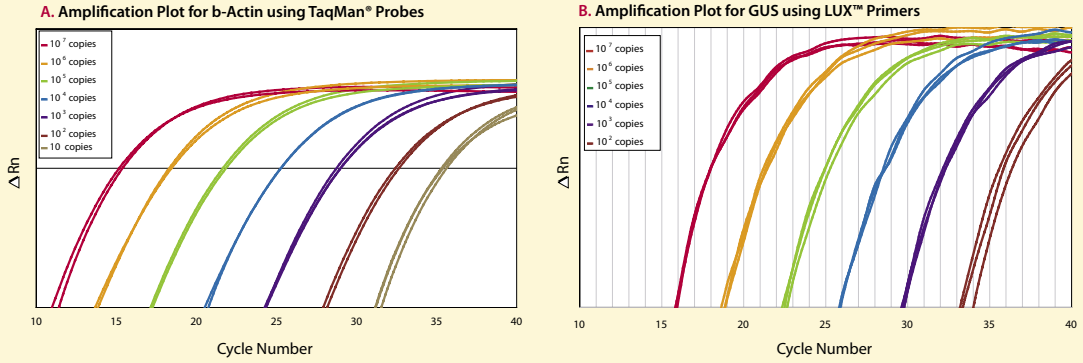
- A simple, robust means of absolute and standard curve quantification
- An easy way to determine relative copy numbers of genes of interest
- Considerable cost and time savings by eliminating the need to produce your own standards
- Reproducible, accurate normalization for experiment-to-experiment and lab-to-lab comparison of qPCR data (Figure 37)

Figure 36 —qPCR Plasmid Standards provide accurate normalization for both high-abundance (A) and medium-low-abundance (B) genes



1x10^7 copies of qPCR Plasmid Standards, High Abundance (ß Actin, B2M, GAPDH, and RPL4; Panel A) and qPCR Plasmid Standards, Medium-Low Abundance (GUS, HMBS, HPRT, and PPIA; Panel B) were amplified with Certified LUX™ Primer Sets labeled with FAM. All reactions were performed on the ABI PRISM® 7700.

Figure 37 — qPCR Plasmid Standards provide reliable normalization over a broad dynamic range



Panel A: TaqMan® primers and probe for β-Actin labeled with FAM-TAMRA used to detect 1x10^7 to 10 copies of the qPCR Plasmid Standards, High Abundance on the Corbett Rotor-Gene™. **Panel B:** Certified LUX™ Primers for GUS labeled with FAM used to detect 1x10^7 to 100 copies of the qPCR Plasmid Standards, Medium-Low Abundance on the ABI PRISM® 7700.

Product	Quantity	Cat. no.
qPCR Plasmid Standards, High Abundance	100 µl	11740-100
qPCR Plasmid Standards, Medium-Low Abundance	100 µl	11741-100

Related products

Product	Quantity	Cat. no.
qPCR accessories		
Fluorescein Reference Standard	5 x 1 ml	F36915
ROX Reference Dye	500 µl	12223-012
Uracil DNA Glycosylase (1 U/µl)	100 units	18054-015
DNA purification		
Plasmid DNA Purification	visit www.invitrogen.com/nap	
Genomic DNA Purification	visit www.invitrogen.com/nap	
DNA Clean-Up	visit www.invitrogen.com/nap	
RNA purification		
ChargeSwitch® Total RNA Cell Kit	50 preps	CS14010
PureLink™ 96 Total RNA Purification Kit	4 x 96 preps	12173-011
Micro-to-Midi™ Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml	15596-026
RNA sample QC		
Quant-iT™ RNA Assay Kit	1000 assays	Q33140
RiboGreen® RNA Quantitation Kit	200-2000 assays	R11490
Microarray labeling and detection		
SuperScript™ RNA Amplification System	20 rxns	L1016-01
SuperScript™ Indirect RNA Amplification System	20 rxns	L1016-02
SuperScript™ Direct cDNA Labeling System	30 rxns	L1015-02
SuperScript™ Plus Direct cDNA Labeling System dUTP	30 rxns	L1015-06
SuperScript™ Indirect cDNA Labeling System	30 rxns	L1014-02
SuperScript™ Plus Indirect cDNA Labeling System dUTP	30 rxns	L1014-06
RNAi products		
Validated Stealth™ RNAi DuoPaks	visit www.invitrogen.com/RNAi	
Stealth™ Select RNAi	visit www.invitrogen.com/RNAi	
Custom BLOCK-iT™ siRNA	visit www.invitrogen.com/RNAi	
BLOCK-iT™ Vectors	visit www.invitrogen.com/RNAi	

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