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Sensitive, specific real-time PCR without probes

LUX™ Fluorogenic Primers are:

- **Sensitive**—broad dynamic range for detection of low-abundance genes
- **Specific**—sequence-specific detection with option for melting curve analysis
- **Cost-effective**—economical alternative to dual-labeled fluorogenic probes

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Sensitive, specific, and economical real-time detection

LUX™ Fluorogenic Primers provide an innovative detection method for real-time PCR/RT-PCR. Using a custom-designed, single-labeled primer, you can achieve highly specific and sensitive quantification of your gene of interest, cost-effectively.

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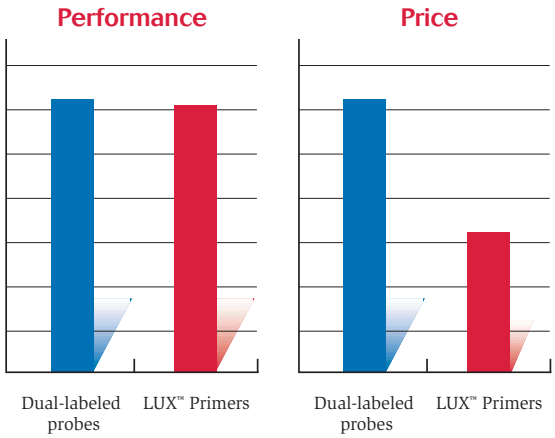
Fluorogenic primer-based quantitative PCR

Real-time PCR/RT-PCR based on fluorogenic detection has become the technology of choice for accurate, reproducible quantification of DNA and RNA. LUX™ Fluorogenic Primers present a high-performing yet cost-effective alternative to dual-labeled fluorogenic probe methods. Each LUX™ Primer Set[114] includes a single-labeled fluorogenic primer and a corresponding unlabeled primer. They provide a complete primer set for PCR and offer real-time detection without the need for probes or quenchers. Using the LUX™ platform, you get the performance and analysis capabilities you need—high specificity and sensitivity, broad dynamic range, multiplexing, melting curve analysis, and simple design—all at about half of the cost of probe-based technology (Figure 1).

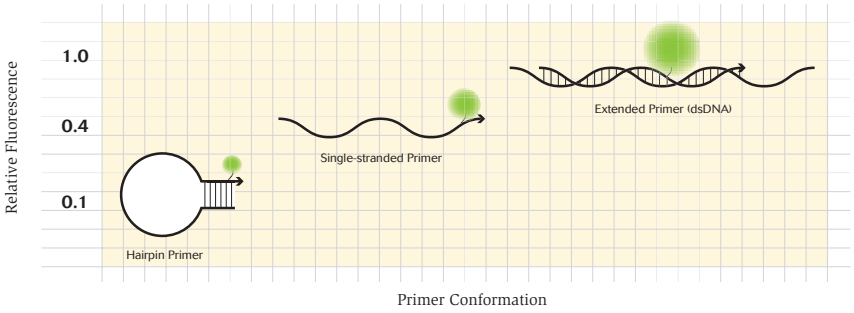
Figure 1 - LUX™ Primers vs. dual-labeled probes



Novel detection mechanism

The LUX™ (Light Upon eXtension) effect presents a novel fluorescent detection mechanism for real-time analysis. LUX™ Primers are oligonucleotides labeled with a single fluorophore, custom-synthesized according to the DNA/RNA of interest. Typically 20-30 bases in length, they are designed with the fluorophore close to the 3´ end in a hairpin structure. This configuration, an advancement from the dual-labeled probe format, intrinsically renders fluorescence quenching capability so that a separate quenching moiety is not needed. When the primer is incorporated into the double-stranded PCR product, the fluorophore is dequenched, resulting in a significant increase in fluorescent signal (Figure 2). This signal increase is the basis for the LUX™ detection platform.

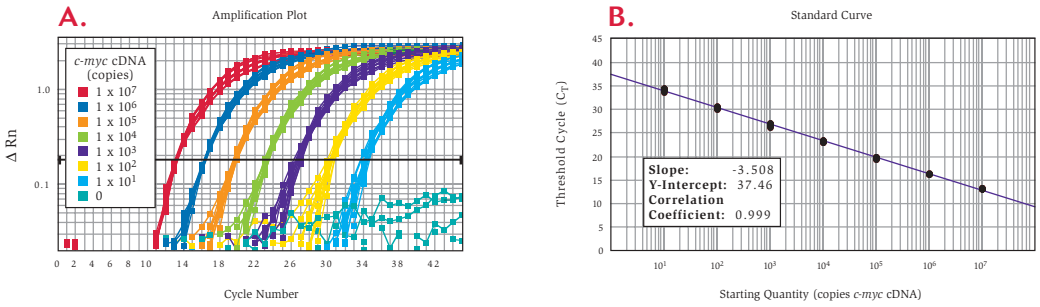
Figure 2 - The LUX™ (Light Upon eXtension) effect



High-performance real-time PCR and RT-PCR

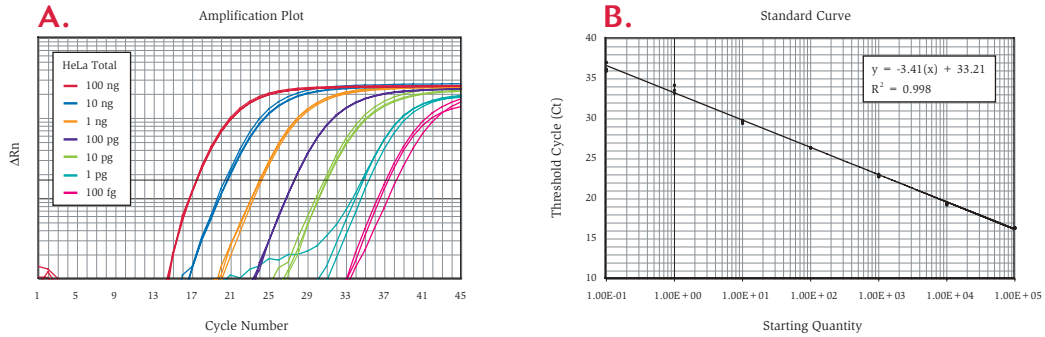
The LUX™ platform gives you the performance you need to achieve the best real-time quantification results. It routinely detects 100 or fewer copies of target genes, measures picogram amounts of DNA/RNA, and achieves a dynamic range of 7 orders in magnitude (Figures 3 and 4). The sensitivity and specificity of LUX™ detection is comparable to dual-labeled probe-based detection using such products as TaqMan® Probes or Molecular Beacons.

Figure 3 - Real-time PCR of *c-myc* cDNA



Panel A: Real-time PCR of serial dilutions of a *c-myc* cDNA clone were performed using 200 nM FAM-labeled LUX™ Primer, 200 nM unlabeled primer, Platinum® Quantitative PCR SuperMix-UDG, and ROX Reference Dye. Reactions were incubated 3 min at 95°C, followed by 45 cycles of 95°C, 15s; 60°C, 45s using an ABI PRISM® 7700. **Panel B:** Standard curve showing the starting template amount versus C_t value.

Figure 4 - Real-time RT-PCR of HeLa total RNA

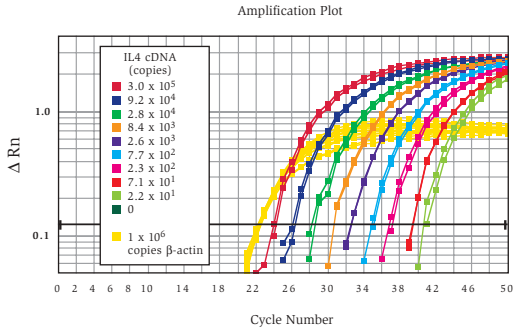


Panel A: The human β-actin transcript was quantified in samples comprising 10-fold serial dilutions of HeLa cell total RNA ranging from 100 ng to 0.1 pg in triplicates including no template controls. The one-step RT-PCR in real-time was carried with 200 nM FAM-labeled LUX™ Primer (forward), 200 nM unlabeled primer (reverse), Platinum® Quantitative RT-PCR ThermoScript™ One-Step System, and ROX Reference Dye. Reactions were incubated 30 min at 50°C (RT reaction) followed by 5 min at 95°C and 45 cycles of 95°C for 15s/60°C for 45s (PCR step) using an ABI PRISM® 7700. **Panel B:** Standard curve showing the starting template amount versus C_t value.

Efficient multiplexing

Multiplexing enables you to profile multiple genes in a single sample. Using dual-labeled probe technology, the process requires monitoring two wavelengths per amplicon—one for the fluorophore, the other for the quencher. Binding dyes like SYBR® Green I lack any multiplexing capability. With LUX™ Primers, multiplexing is not only possible, it's also simple and efficient. Since you use a single-labeled primer, you monitor just one fluorescent label per target. LUX™ Primers are available with either FAM or JOE fluorophors. Figure 5 demonstrates highly efficient multiplex amplification of the IL-4 gene with a FAM-labeled LUX™ primer and β-actin gene with a JOE-labeled LUX™ primer.

Figure 5 - Multiplexed PCR of IL-4 cDNA and β-actin

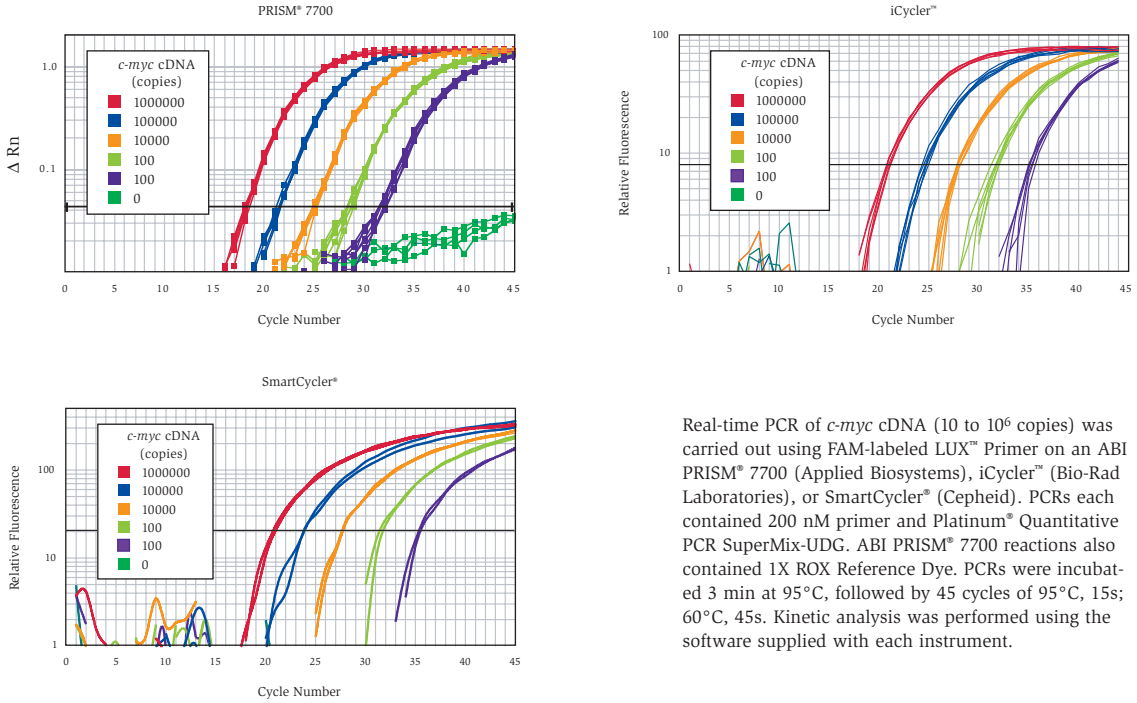


Serial dilutions of a human IL-4 cDNA clone were amplified using Platinum® Quantitative PCR SuperMix-UDG in 50 µl volumes with 200 nM FAM-labeled LUX™ Primer, 200 nM reverse primer, and ROX Reference Dye. PCRs were cycled for 45 cycles of: 95°C, 15s; 55°C, 30s; 72°C, 30s on an ABI PRISM® 7700 sequence detection system. FAM fluorescent signal collected at the extension step (normalized against ROX Reference Dye) was used for kinetic PCR analysis. Multiplexed PCRs were performed for 50 cycles as described above with the inclusion of 1 x 10^6 copies of a β-actin cDNA clone and 200 nM JOE-labeled LUX™ Primer set for β-actin.

Compatible with multiple instruments

With LUX™ Primers, you are not restricted to any particular instrument platform. LUX™ Primers are compatible with a wide variety of real-time machines, including the ABI PRISM® 7700/7000/7900 and GeneAmp® 5700, Bio-Rad iCycler™, Stratagene Mx4000™, Cepheid SmartCycler®, and Corbett Research Rotor-Gene™. Whatever instrument you use, you'll get excellent results (Figure 6). Validated protocols for using LUX™ Primers can be found at www.invitrogen.com/lux.

Figure 6 - Validation of LUX™ Primers on various real-time instruments



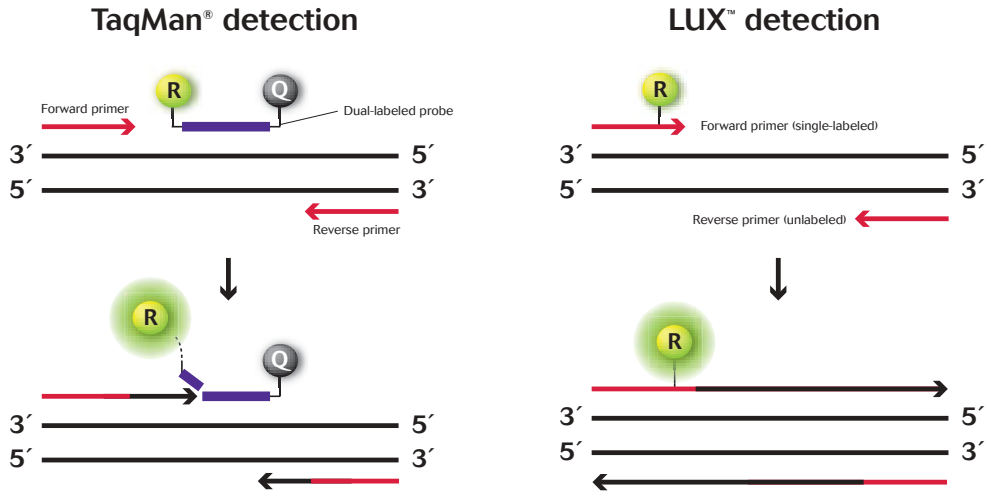
Real-time PCR of *c-myc* cDNA (10 to 10^6 copies) was carried out using FAM-labeled LUX™ Primer on an ABI PRISM® 7700 (Applied Biosystems), iCycler™ (Bio-Rad Laboratories), or SmartCycler® (Cepheid). PCRs each contained 200 nM primer and Platinum® Quantitative PCR SuperMix-UDG. ABI PRISM® 7700 reactions also contained 1X ROX Reference Dye. PCRs were incubated 3 min at 95°C, followed by 45 cycles of 95°C, 15s; 60°C, 45s. Kinetic analysis was performed using the software supplied with each instrument.

The smart alternative to probe technology

Dual-labeled fluorogenic probes such as TaqMan® Probes or Molecular Beacons each require two fluorescent dyes: one reporter (R) and one quencher (Q). The quencher reduces the fluorophore signal when the two moieties are in close proximity. These dual-labeled oligonucleotides are difficult to design and expensive to produce.

The LUX™ platform provides a smart 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 LUX™ platform, all you need is one fluorogenic primer labeled with a single reporter (R) dye, and one corresponding unlabeled primer (Figure 7). The fluorogenic primer can be either forward or reverse. The result: design is simple and production is fast and inexpensive, allowing you to analyze more genes at about half the cost of TaqMan® Probes.

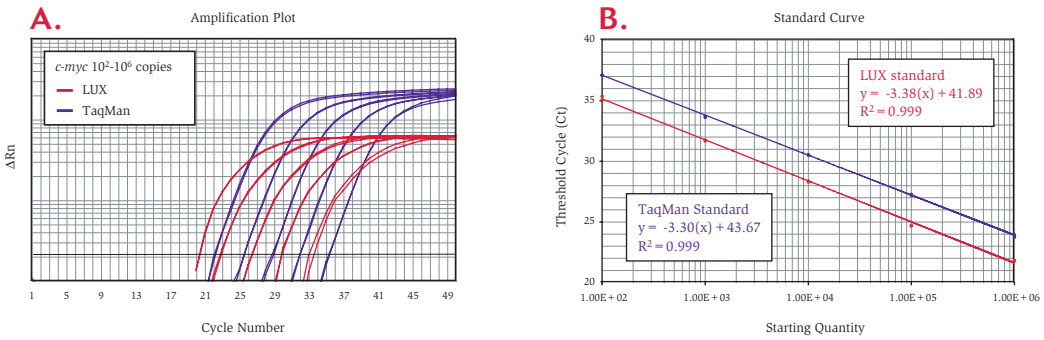
Figure 7 - Comparison of dual-labeled probe and single-labeled LUX™ Primer detection



The smart alternative to probe technology, continued

More importantly, you achieve comparable sensitivity and specificity, as demonstrated by the similar C_t values obtained with both systems (Figure 8). The LUX™ platform also enables you to perform melting curve analysis, a powerful tool to identify primer-dimer artifacts and an option not available with probe-based detection.

Figure 8 - Amplification plots comparing LUX™ Primer and TaqMan® Probe



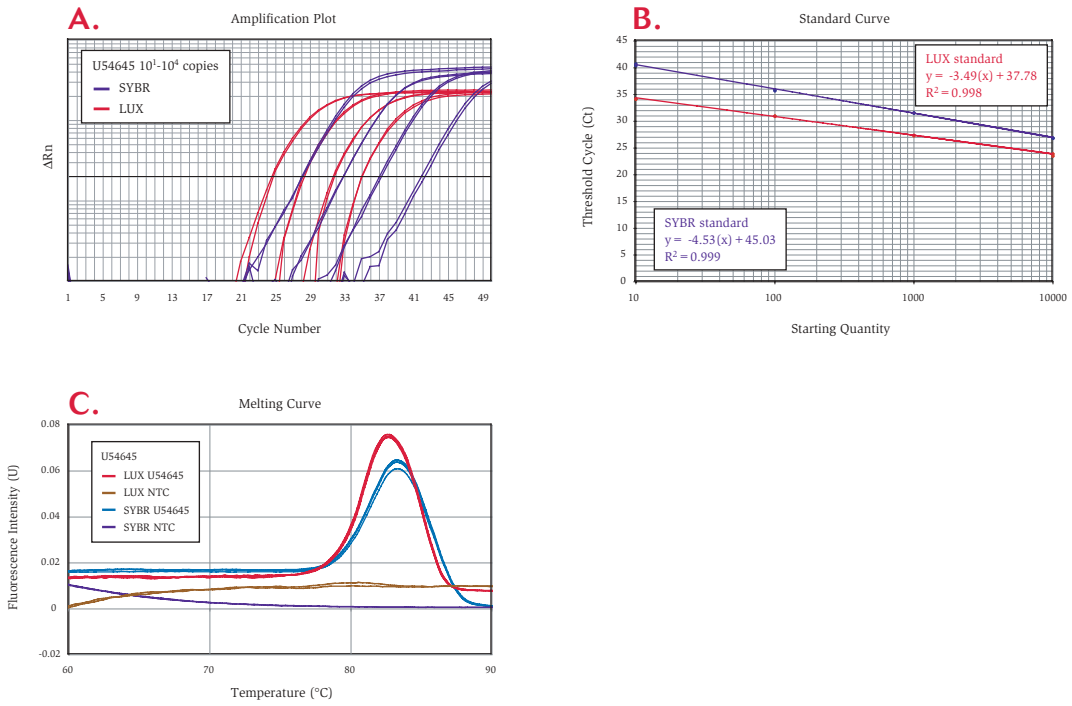
Panel A: Real-time PCR of 10 to 10^5 copies of *c-myc* cDNA was performed using 200 nM FAM-labeled LUX™ Primer, 200 nM unlabeled primer, and Platinum® Quantitative PCR SuperMix-UDG with ROX Reference Dye. TaqMan® Universal PCR Master Mix was used to amplify the same template with 200 nM unlabeled primers and 100 nM TaqMan® Probes. Both LUX™ Primers and TaqMan® Probes targeted the same region of the *c-myc* gene. Reactions were incubated 2 min at 50°C, then for 10 min at 95°C, followed by 50 cycles of 95°C, 15s; 60°C, 45s using an ABI PRISM® 7700.
Panel B: Standard Curve showing the starting template amount versus C_t value.

More reliable than DNA binding dyes

Double-stranded DNA binding dyes such as SYBR® Green I are commonly used as a low-cost alternative to probe technology for real-time applications. The fluorescence generated is proportional to the amount of product accumulated. Although inexpensive, SYBR® Green I binds indiscriminately to DNA, detecting PCR artifacts such as primer-dimers and spurious amplification products. The nonspecific nature of the reaction and lack of multiplexing ability are major drawbacks of this method.

Because LUX™ Primers are designed specifically for your target sequence, you obtain more reliable data. Figure 9 shows that LUX™ is significantly more sensitive than SYBR® Green I and achieves the C_t value 5 cycles earlier. You can use different fluorescent labels with LUX™ Primers for multiplex applications, a capability not available with DNA binding dyes. In addition, just like SYBR® Green I you can perform melting curve analysis using LUX™ Primers. This tool enables you to distinguish bona fide amplicons from primer-dimer artifacts (Figure 9).

Figure 9 - Amplification plots comparing LUX™ Primer and SYBR® Green I dye

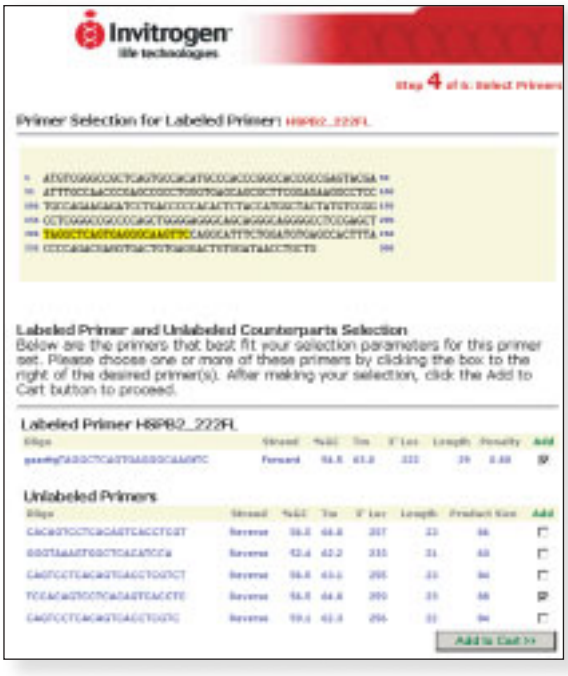


Panel A: Real-time PCR of 10 to 10^4 copies of adenylate kinase 2 was performed using 200 nM FAM-labeled LUX™ Primer, 200 nM unlabeled primer, and Platinum® Quantitative PCR SuperMix-UDG with ROX Reference Dye. A commercially available SYBR® Green I qPCR 2x mix with ROX Reference Dye was used to amplify the same template with 200 nM unlabeled primers. Both primer sets targeted the same region of the adenylate kinase 2 gene. Reactions were done on the same plate and were incubated 2 min at 50°C, then for 10 min at 95°C, followed by 50 cycles of 95°C, 15s; 60°C, 45s using an ABI PRISM® 7700. Melting curve analysis was performed for both LUX™ and SYBR® Green detection. **Panel B:** Standard Curve showing the starting template amount versus C_t value. **Panel C:** Melting curve analysis was performed for both LUX™ and SYBR® Green detection.

Simple primer design

You can design LUX™ Primer Sets using LUX™ Designer, our web-based, user-friendly primer design software, at www.invitrogen.com/lux. Just enter the gene sequence of interest, and the software will generate one or more primer sets ranked in order of optimization. Each primer set includes a fluorogenic primer and a corresponding unlabeled primer. The primer sets are designed to provide the best possible amplicon size and to minimize primer-dimer formation. Choose a set and order directly online from the Invitrogen Custom Primer Facility. It's that easy (Figure 10).

Figure 10 - LUX™ Designer software



Quality manufacturing

All LUX™ Primers are manufactured according to your design specifications and rapidly delivered. We incorporate a number of quality control checks throughout our proprietary synthesis process to ensure that you get primers of the highest quality. Your primers are delivered within 5-7 days after you order online. With each order you'll receive the following components:

- Fluorogenic primer (available with FAM or JOE label, in 50 nmol or 200 nmol scale, supplied lyophilized)
- Corresponding unlabeled primer, supplied lyophilized
- Certificate of Analysis (COA) containing information on the name, sequence, label, and quantity of each primer

In addition, you can download the user manual for LUX™ Primers, in PDF format, at www.invitrogen.com/lux. It contains detailed protocols for using LUX™ Primers in real-time PCR/RT-PCR applications.

High-performing real-time reagents

To achieve the best results in your real-time applications, combine LUX™ Fluorogenic Primers with the leading quantitative PCR reagents and systems optimized for real-time applications (Table 1). Invitrogen qPCR products are based on Platinum® *Taq* hot-start technology and RNAse H minus reverse transcriptases. These enzyme mixtures provide superior performance, broad dynamic range, high sensitivity and specificity, and convenient product configuration. You'll achieve reliable, consistent results in your quantitative analysis, experiment after experiment.

Table 1 - Invitrogen products for real-time PCR/RT-PCR

Product	Application	Reactions	Cat. no.
Platinum® Quantitative PCR SuperMix-UDG[2,9,12,14,24]	A mastermix optimized for qPCR. Combines the specificity of Platinum® *Taq* with UDG decontamination	100 rxns 500 rxns	11730-017 11730-025
SuperScript™ One Step RT-PCR System with Platinum® *Taq* DNA Polymerase[1,2,5,14]	One-step RT-PCR combining SuperScript™ II RT with Platinum® *Taq*, with options for both endpoint and real-time detection	25 rxns 100 rxns	10928-034 10928-042
Platinum® Quantitative RT-PCR ThermoScript™ One-Step System[2,13,14,24]	One-step qRT-PCR combining ThermoScript™ RT with Platinum® *Taq*. Optimal for high-temperature cDNA synthesis on difficult templates; formulated for real-time analysis	100 rxns 500 rxns	11731-015 11731-023
SuperScript™ First Strand Synthesis System for RT-PCR[4,5]	Used for cDNA synthesis. Follow with Platinum® Quantitative PCR SuperMix-UDG for two-step qRT-PCR	50 rxns	11904-018
ROX Reference Dye[4]	Passive reference for fluorescence normalization	500 µl	12223-012

Discover the power of LUX™

Compared to dual-labeled probes or DNA binding dyes, LUX™ Fluorogenic Primers present an attractive alternative for real-time PCR detection. The LUX™ platform combines superior performance with superior cost-effectiveness (Table 2). This is the detection technology you have been waiting for. Visit www.invitrogen.com/lux today—and discover the power of LUX™.

Table 2 - 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	•	•	• • •	• • •

Description†	Quantity
LUX™ Fluorogenic Primer Set, FAM-labeled*	50 nmol
	200 nmol
LUX™ Fluorogenic Primer Set, JOE-labeled*	50 nmol
	200 nmol

† Design and order LUX™ Fluorogenic Primer sets online at www.invitrogen.com/lux.

LUX™ Fluorogenic Primers are designed using a proprietary synthesis methodology, and have been validated using Invitrogen's quantitative PCR reagents (Table 1, page 11).

References:
Nazarenko, I. *et al.* (2002) *Nucleic Acids Research* **30:** e37.
Nazarenko, I. *et al.* (2002) *Nucleic Acids Research* **30:** 2089-2095.

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