Invitrogen™
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Instruction Manual

LUX™ Fluorogenic Primers

For real-time PCR and RT-PCR

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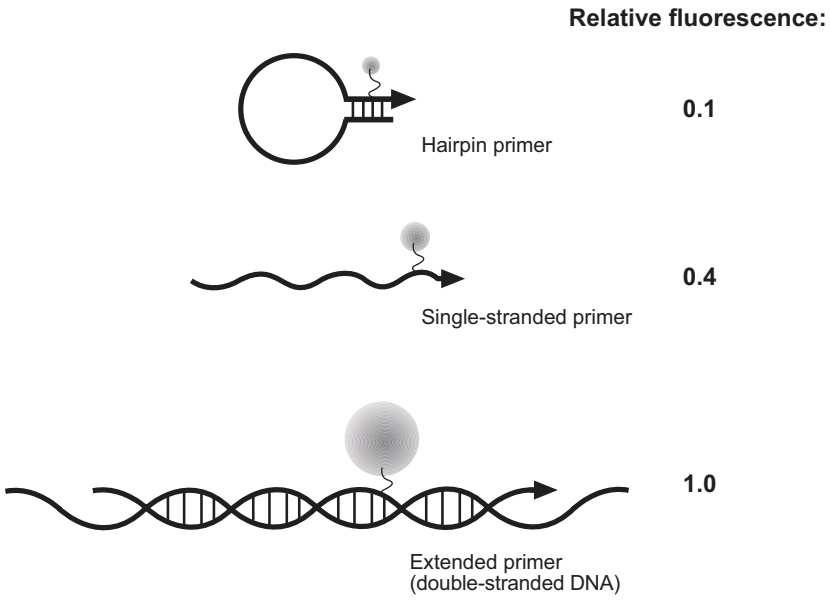
Introduction

Overview

LUX™ (Light Upon Extension) primers are an easy to use, highly sensitive, and efficient method for performing real-time ("quantitative") PCR and RT-PCR. LUX™ primers combine high specificity and multiplexing capability with simple design and streamlined protocols. LUX™ primers are compatible with melting curve analysis of real-time PCR products, allowing the differentiation of amplicons and primer dimer artifacts by their melting temperatures. LUX™ primers require no special probes or quenchers, and can be ordered direct from Invitrogen.

Each primer pair in the LUX™ system includes a fluorogenic primer with a fluorophore attached to its 3′ end, as well as a corresponding unlabeled primer. The fluorogenic primer has a short sequence tail of 4–6 nucleotides on the 5′ end that is complementary to the 3′ end of the primer. The resulting hairpin secondary structure provides optimal quenching of the fluorophore (see the figure below). When the primer is incorporated into the double-stranded PCR product, the fluorophore is dequenched and the signal increases by up to 10-fold.

LUX™ Primer Reaction



Labeling

Each fluorogenic LUX™ primer is labeled with one of two reporter dyes—FAM (6-carboxy-fluorescein) or JOE (6-carboxy-4', 5'-dichloro-2', 7'-dimethoxy-fluorescein). Additional reporter dyes will be available in the future.

A multiplex real-time reaction contains different primer sets, each with a different label, to detect different genes in the same sample.

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Applications

LUX™ primers can be used in real-time PCR and RT-PCR to quantify 100 or fewer copies of a target gene in as little as 1 pg of template DNA or RNA. LUX™ primers have a broad dynamic range of 7–8 orders.

Multiplex applications use separate FAM and JOE-labeled primer sets to detect two different genes in the same sample. Typically, the FAM-labeled primer is used to detect the gene of interest and the JOE-labeled primer is used to detect a housekeeping gene used as an internal control.

Instrument Compatibility

LUX™ primers are compatible with a wide variety of real-time PCR instruments, including but not limited to the ABI PRISM® 7700/7000/7900 and GeneAmp® 5700, the Bio-Rad iCycler™, the Stratagene Mx4000™, and the Cepheid Smart Cycler®.

ABI PRISM is a registered trademark of Applera Corporation. GeneAmp is a registeredtrademark of Roche Molecular Systems, Inc. iCycler, Mx4000, and Smart Cycler are trademarks of their respective companies.

Designing and Ordering Primers

LUX™ Designer Web-based Design Software

To design and order LUX™ primers, visit the Invitrogen LUX™ Web site at www.invitrogen.com/LUX and follow the link to the LUX™ Designer Web-based design software. Follow the step-by-step instructions in the software to submit target sequences containing your genes of interest and generate primer designs. At any point in the process, click on the LUX™ Designer **Help** button for more detailed instructions and assistance.

LUX™ Designer will automatically generate one or more primer designs based on each sequence you submit and the selected design parameters. The design software includes algorithms to minimize primer self-complementarity and interactions between primers. It also assigns rankings to the generated designs—based on primer melting temperature, hairpin structure, self-annealing properties, etc.—to aid in selection.

When the designs have been generated, you can review them, select a design, select the fluorophore labels, and place your order.

Submitting a Target Sequence

When you submit a target sequence containing your gene of interest, keep in mind the following design criteria:

- The optimal amplicon length for real-time PCR ranges from 80 to 200 bases. You can specify a minimum, optimal, and maximum amplicon length when you submit the sequence.
- The target sequence should be at least 10 bases longer than the minimum amplicon size you select. **The longer the sequence, the more likely that an optimal primer design can be developed**.
- The sequence must contain only standard IUPAC (International Union of Pure and Applied Chemistry) letter abbreviations.
- When you first submit a sequence, the Disable Penalty Checking checkbox should *not* be checked; the resulting penalty scores provide an important measure of primer suitability. **Penalty scores in the range of 0.0–4.0 are acceptable.** If no primers with a penalty score of 4.0 or lower are generated, you may choose to disable Penalty Checking and redesign the primers. Note that if you select a primer with a higher penalty score, the efficiency of the reaction may be less than optimal. See the LUX™ Designer **Help** for guidance in optimizing your design parameters and/or sequence.
- When you select the design parameters, the default melting temperature range is 60–68ºC. Do not change this default unless the design engine finds no primers in this range. For primers in this range, PCR programs with annealing temperatures from 55º to 64ºC are appropriate.

Selecting a Primer Design

After you submit your sequence, LUX™ Designer will first generate one or more designs for the labeled primer. The labeled primer can be either the forward or the reverse primer (note the recommendation for one-step RT-PCR on the following page). After you select a design for the labeled primer, you will be prompted to select a design for the corresponding unlabeled primer.

Continued on next page

In one-step RT-PCR (see the sample reaction on page 11), the reverse primer drives the reverse transcription reaction. We have found that labeling the reverse primer with the LUX™ fluorophore can inhibit this RT step; therefore, we strongly recommend that you select the **forward primer as the labeled primer**. You should choose a forward primer as the labeled primer for one-step RT-PCR regardless of its penalty score.

If no forward primer can be generated for the sequence, you may select the reverse primer as the labeled primer, noting that the efficiency of the reaction may be compromised.

Selecting Labels

After you have selected a primer set (labeled and unlabeled) for a particular sequence, you can specify the particular label and synthesis scale

When selecting labels in a multiplex reaction, we recommend using the FAM label for your gene of interest and the JOE label for the housekeeping gene that you will use as the internal control.

Placing the Order

After you have selected the label and synthesis scale, you can submit your order directly from the LUX™ Designer Web site. Each primer order will be shipped directly from Invitrogen's Custom Primer Facilities. Labeled and unlabeled primers are shipped separately. Labeled primers are shipped in an amber tube; unlabeled primers are shipped in a clear tube.

Each primer ordered from Invitrogen's Custom Primer Facilities comes with a Certificate of Analysis (COA) verifying the amount and sequence.

Product Qualification

LUX™ primers are provided in 50 nM or 200 nM synthesis scale. They are tested post-synthesis by optical density (OD) ratio measurements and mass spectroscopy to ensure efficient dye labeling and correct molecular weight and composition.

See the Certificate of Analysis shipped with each primer for more information.

Storing and Reconstituting Primers

Primer Storage and Stability

Store primers at –20ºC in the dark. Primers are stable for:

- >12 months when stored at –20ºC in lyophilized form.
- >6 months when stored at –20ºC in solution.

Stability can be extended by storing at –70ºC.

Reconstituting Primers

LUX™ primers are provided in 50 nmole or 200 nmole synthesis scale. To reconstitute primers, centrifuge the tube for a few seconds to collect the oligonucleotide in the bottom of the tube. Carefully open, add an appropriate volume of TE buffer or ultrapure water, close the tube, rehydrate for 5 minutes, and vortex for 15 seconds.

We recommend that you rehydrate primers at concentrations greater than 10 µM. To prepare a 100 µM primer stock solution, multiply the primer amount in nmoles by ten to determine the volume of diluent in µl.

After reconstitution, store the primer stock at –20ºC in the dark, where it will be stable for 6 months or more.

Real-Time PCR

Introduction

This section provides guidelines and an optimized protocol for performing real-time PCR using LUX™ primers.

Template Specifications

The target template for real-time PCR is linear single-stranded or double-stranded DNA, cDNA, or circular DNA (such as plasmids). The amount of DNA typically ranges from 10^2 to 10^7 copies or 1 pg to 10 µg of template.

See page 9 for instructions on generating cDNA using reverse transcription as part of two-step real-time RT-PCR.

Primer Concentration

For optimal PCR conditions, primer titrations of 50–500 nM per primer are recommended. The 50 µl sample reaction on the following page uses 200 nM of each primer, equivalent to 1 µl of a 10 µM primer solution.

Magnesium Concentration

The optimal Mg^{++} concentration for a given target/primer/polymerase combination can vary between 1 mM and 10 mM, but is usually in the range of 3 mM. See the sample reaction on page 7.

dNTP Concentration

The optimal concentration of dATP, dCTP, dGTP, and dTTP is 200 µM each. If dUTP is used in place of dTTP, its optimal concentration is 400 µM.

Instrument Specifications

LUX™ primers are compatible with a wide variety of real-time PCR instruments with various detection capabilities, including but not limited to the ABI PRISM® 7700/7000/7900 and GeneAmp™ 5700, the Bio-Rad iCycler™, the Stratagene Mx4000™, and the Cepheid Smart Cycler®.

At a minimum, the instrument used to perform real-time PCR with LUX™ primers must be able to:

- Detect fluorescence at each PCR cycle
- Excite FAM- and JOE-labeled LUX™ primers near their excitation wavelength maximums of 490 nm and 520 nm, respectively
- Detect the emission of FAM and JOE-labeled LUX™ primers near their emission maximums of 520 nm and 550 nm, respectively

Please refer to the specific instrument's user manual for operating instructions.

Instrument Settings

Please follow the manufacturer's instructions for configuring your real-time PCR instrument for use with LUX™ primers. Note the following settings:

- Because LUX™ primers do not contain a quencher, the quencher setting should be set to **no quencher**.
- We recommend the use of ROX reference dye (Catalog no. 12223-023) for normalization of well-to-well variation. Adjust your instrument reference setting accordingly.

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Enzyme Specifications

We recommend using a "hot-start" DNA polymerase, preferably one that has been optimized for real-time PCR. Platinum® Quantitative PCR SuperMix-UDG, available from Invitrogen (Catalog no. 11730-017), is a 2X-concentrated, ready-to-use reaction mixture containing all components except primers and template. It uses Platinum® *Taq* DNA polymerase, and has been specifically formulated to provide optimal performance in real-time PCR systems. See the sample protocol below.

Real-Time PCR Protocol

The following optimized reaction uses Platinum® Quantitative PCR SuperMix-UDG with ROX reference reagent.

Note: Before proceeding, see the real-time PCR guidelines on the previous page. For multiplex reactions, see the guidelines on the following page.

1. To reduce pipetting errors, prepare a Master Mix of all the reaction ingredients except template. The following table provides Master Mix volumes for one reaction and 50 reactions (scale up or down as needed):

Component	Stock conc	Vol: 1 rxn	Vol: 50 rxns	Rxn conc
Platinum® Quantitative PCR SuperMix-UDG*	2X	25 µl	1250 µl	1 X
ROX reference dye	50X	1 µl	50 µl	1 X
Sterile distilled H_2O**	—	12 µl	600 µl	—
Reverse primer	10 µM	1 µl	50 µl	200 nM
Forward primer	10 µM	1 µl	50 µl	200 nM
Total volume	—	40 µl	2000 µl	—

*Supplied at 2X concentration: 60 U/ml Platinum® *Taq* DNA polymerase, 40 mM Tris-HCl (pH 8.4), 100 mM KCl, 6 mM $MgCl_2$, 400 µM dGTP, 400 µM dATP, 400 µM dCTP, 800 µM dUTP, 40 U/ml UDG, and stabilizers.

**or use DNase/RNase Free Distilled Water (Cat. No. 10977-015).

2. Program the real-time PCR instrument with one of the following thermal cycling protocols:

3-Step Cycling (recommended)

50°C, 2 min hold
95°C, 2 min hold
45 cycles of:
  95°C, 15 s
  55°C, 30 s
  72°C, 30 s

2-Step Cycling (optional)

50°C, 2 min hold
95°C, 2 min hold
45 cycles of:
  95°C, 15 s
  60-65°C, 30-45 s

3. Add 40 µl of the Master Mix to an optical PCR tube or each well of a 96-well PCR plate.
4. Add 10 µl of template diluted in TE or sterile dH_2O to the tube or each well of the 96-well PCR plate. Cap or seal the tube/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/plate wells. Centrifuge briefly if needed.
6. Place reaction in the real-time PCR instrument and run the program. Collect and analyze results.

Multiplex Real-Time PCR

Multiplex Real-Time PCR

In multiplex real-time PCR, different sets of primers with different labels are used to amplify separate genes on the template DNA. LUX™ primers have been tested in multiplex reactions using FAM to label the gene of interest and JOE to label a housekeeping gene used as an internal control to normalize between different reactions.

In a standard multiplex reaction, you can include the additional primers at the same volumes and concentration as the primers in a singleplex reaction, as shown in the example mixture below:

Component	Volume
Platinum® Quantitative PCR SuperMix-UDG (2X)	25 µl
ROX reference dye (50X)	1 µl
Sterile distilled H_2O	10 µl
Template	10 µl
Forward primer 1 (FAM label) (10 µM)	1 µl
Reverse primer 1 (10 µM)	1 µl
Forward primer 2 (JOE label) (10 µM)	1 µl
Reverse primer 2 (10 µM)	1 µl
Total volume	50 µl

Reduce the volume of water to compensate for the additional primer volume. All other reaction volumes remain the same.

Follow the thermal cycling guidelines provided in the sample reaction on the previous page. If you have difficulty performing the multiplex reaction using these guidelines, see the optimization hints below.

Optimizing Multiplex Conditions

If you notice a decline in real-time PCR efficiency in your multiplex real-time PCR, you can optimize the reaction by performing the steps listed below.

Note: We recommend that you perform one optimization step and then repeat the reaction to test for efficiency before moving on to the next step:

1. Reduce the primer concentration of the gene with the highest abundance (typically the housekeeping gene) to 1/4 the primer concentration of the other gene. For example, in a standard 50 µl reaction, you would add the primers for the less abundant gene at 1 µl each, and add the primers for the more abundant gene at 0.25 µl each.
2. Increase the $MgCl_2$ in the reaction from 3 mM to 5 mM.
3. Increase the dNTP concentrations in the reaction to 400 µM each.
4. Double the amount of polymerase enzyme (to 0.06 U per µl of reaction volume). If you are using Platinum® Quantitative PCR SuperMix-UDG, add Platinum® *Taq* DNA polymerase stand-alone enzyme (Catalog no. 10966-018) to double the amount of enzyme.

Two-Step Real-Time RT-PCR

Introduction

For real-time RT-PCR applications, we recommend a two-step protocol so that the RT and PCR modules can be optimized separately for maximum efficiency and specificity.

This section provides an optimized protocol for performing reverse transcription as part of a two-step real-time RT-PCR protocol. You can use the resulting cDNA in the real-time PCR reaction on pages 6–7.

Template Specifications

The target template for real-time RT-PCR is RNA—usually total cellular RNA or mRNA. The amount of RNA typically varies from 1 pg to 100 ng of template per assay. The purity and integrity of the RNA have a direct impact on results. RNase and genomic DNA contamination are the most common problems, and purification methods should include RNase inhibitors and DNase digestion to minimize these.

We recommend using the Micro-to-Midi Total RNA Purification System (Catalog no. 12183-018) or TRIzol® reagent (Catalog no. 15596-026) to isolate total RNA. High-quality total RNA can be purified from as little as 100 cells up to 10^7 cells or 200 mg of tissue.

To isolate mRNA, we recommend using the FastTrack® 2.0 mRNA Isolation Kit (Catalog no. K1593-02).

Enzyme Specifications

We recommend using a "hot-start" enzyme for the RT reaction, such as SuperScript™ II RT. The following sample protocol uses the SuperScript™ First-Strand Synthesis System for RT-PCR (Catalog no. 11904-018), available from Invitrogen, which includes all components needed for the first-strand synthesis reaction except the RNA.

Removing Genomic DNA from RNA Samples

We recommend that you decrease the genomic DNA content in the RNA sample by performing a digest with DNase I, Amplification Grade (Catalog no. 10868-015), as described below. The DNase I digest is designed for up to 1 µg of RNA; for larger amounts of RNA, increase volumes accordingly.

Combine the following in a tube on ice:

Component	Conc.	Volume
RNA template	—	x µl
DNase reaction buffer	10X	1 µl
DNase I, Amplification Grade	1 U/µl	1 µl
DEPC-treated ddH_20		to 10 µl

1. Incubate at room temperature for 15 min.
2. Incubate at 65°C for 15 min to inactivate the DNase I.

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Reverse Transcription Protocol

The following protocol using the SuperScript™ First-Strand Synthesis System for RT-PCR has been optimized for LUX™ primers. Follow this protocol to generate cDNA, which can then be used in real-time PCR (see pages 6–7).

1. Combine the following kit components in a tube on ice. For multiplex reactions, a master mix without RNA may be prepared:

oligo(dT)12-18 0.5 µg/µl	0.5 µl
Random hexamers 50 ng/µl	0.5 µl
RNA (up to 1 µg)	x µl
10x Buffer	2 µl
25 mM $MgCl_2$	4 µl
10 mM dNTP	1 µl
0.1M DTT	2 µl
RNaseOUT™ (40 U/µl)	1 µl
SuperScript™ II RT (50 U/µl)	1 µl
DEPC-treated ddH_2O	to 20 µl

2. Incubate tube at 25°C for 10 min.
3. Incubate tube at 42°C for 30–50 min.
4. Terminate the reaction at 70°C at 15 min, and then chill on ice.
5. Optional RNaseH treatment: Add 1 µl of RNaseH and incubate at 37°C for 20 min.

Store the reaction at –20°C. Use 2–8 µl of cDNA for real-time PCR using Platinum® Quantitative PCR SuperMix-UDG, as described on pages 6–7.

One-Step Real-Time RT-PCR

Introduction

This section provides information and a generic protocol for performing one-step real-time RT-PCR using LUX™ primers. Note that one-step RT-PCR is a complex reaction that may require additional optimization and evaluation of several primer pairs to obtain optimum sensitivity and specificity.

In one-step RT-PCR, the reverse primer drives the reverse transcription reaction. We have found that labeling the reverse primer with the LUX™ fluorophore can inhibit this RT step; therefore, we strongly recommend that you **label the forward primer** with the FAM or JOE fluorophore. See pages 3-4 for guidance on primer design.

Note

LUX™ primers have not been tested in multiplex one-step real-time RT-PCR.

Template Specifications

The target template for one-step real-time RT-PCR is RNA—usually total cellular RNA or mRNA. The amount of template typically ranges from 1 pg to 100 ng per assay. The purity and integrity of the RNA have a direct impact on results. RNase and genomic DNA contamination are the most common problems, and purification methods should be designed to avoid these.

We recommend using the Micro-to-Midi Total RNA Purification System (Catalog no 12183-018) or TRIzol® reagent (Catalog no. 15596-026) to isolate total RNA. High-quality total RNA can be purified from as little as 100 cells up to 10^7 cells or 200 mg of tissue.

To isolate mRNA, we recommend using the FastTrack® 2.0 mRNA Isolation Kit (Catalog no. K1593-02).

Enzyme Specifications

The one-step RT-PCR enzyme mix should be optimized for real-time PCR. We recommend using the Platinum® Quantitative RT-PCR ThermoScript™ One-Step System (Catalog no. 11731-015), which uses a ThermoScript™ Plus/Platinum® *Taq* enzyme mix. It has been optimized for use in real-time fluorescent PCR systems. See the sample reaction on page 13.

Primer Concentration

For optimal PCR conditions, primer titrations of 50–500 nM per primer are recommended. The 50 µl sample reaction on page 13 uses 200 nM of each primer, equivalent to 1 µl of a 10 µM primer solution.

Magnesium Concentration

The optimal Mg^{++} concentration for a given target/primer/polymerase combination can vary between 1 mM and 10 mM, but is usually in the range of 3 mM (see the sample reaction on page 13).

dNTP Concentration

The optimal concentration of dATP, dCTP, dGTP, and dTTP is 200 µM each. If dUTP is used in place of dTTP, its optimal concentration is 400 µM.

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Instrument Specifications

LUX™ primers are compatible with a wide variety of real-time PCR instruments with various detection capabilities, including but not limited to the ABI PRISM® 7700/7000/7900 and GeneAmp™ 5700, the Bio-Rad iCycler™, the Stratagene Mx4000™, and the Cepheid Smart Cycler®.

At a minimum, the instrument used to perform one-step real-time RT-PCR with LUX™ primers must be able to:

- Detect fluorescence at each PCR cycle
- Excite FAM and JOE-labeled primers near their excitation wavelength maximums of 490 nm and 520 nm, respectively
- Detect the emission of FAM and JOE-labeled LUX™ primers near their emission maximums of 520 nm and 550 nm, respectively

Please refer to the specific instrument's user manual for operating instructions.

Instrument Settings

Please follow the manufacturer's instructions for configuring your real-time PCR instrument for use with LUX™ primers. Use the following settings for performing one-step real-time RT-PCR:

- Because LUX™ primers do not contain a quencher, the quencher setting should be set to **no quencher**.
- We recommend the use of ROX reference dye (Catalog no. 12223-023) for normalization of well-to-well variation. Adjust your instrument reference setting accordingly.
- Program the instrument to perform cDNA synthesis immediately followed by PCR amplification.

Removing Genomic DNA from RNA Samples

We recommend that you decrease the genomic DNA content in the RNA sample by performing a digest with DNase I, Amplification Grade (Catalog no. 10868-015), as described below. The DNase I digest is designed for up to 1 µg of RNA; for larger amounts of RNA, increase volumes accordingly.

Combine the following in a tube on ice:

Component	Conc.	Volume
RNA template	—	x µl
DNase reaction buffer	10X	1 µl
DNase I, Amplification Grade	1 U/µl	1 µl
DEPC-treated ddH_20		to 10 µl

6. Incubate at room temperature for 15 min.
7. Incubate at 65°C for 15 min to inactivate the DNase I.

The absence of genomic DNA in the RNA sample can be verified by preparing a control reaction identical to the reaction on the following page, using 2 U of Platinum® *Taq* DNA polymerase (Catalog no. 10966-018) in place of the ThermoScript™ Plus/Platinum® *Taq* Enzyme Mix.

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One-Step Real-Time RT-PCR Protocol

The following protocol using Platinum® Quantitative RT-PCR ThermoScript™ One-Step System has been optimized for LUX™ primers. Because one-step RT-PCR is a complex reaction that is susceptible to artifacts, further optimization may be required.

Note: Keep all components, reaction mixes and samples on ice. After assembly, transfer the reaction to a thermal cycler preheated to the desired cDNA synthesis temperature (50-70°C) and immediately begin RT-PCR. We recommend performing the cDNA synthesis reaction at 50ºC, but higher temperatures (up to 70ºC) may be required for high GC content templates.

RNase inhibitor proteins, such as RNaseOUT™ (Catalog no. 10777-019), may be added to the reaction following the addition of the 2X ThermoScript reaction mix to safeguard against degradation of RNA.

1. To reduce well-to-well variation, prepare a Master Mix of all the reaction ingredients except RNA. The following table provides Master Mix volumes for one reaction and 50 reactions (scale up or down as needed):

Component	Stock conc	Vol: 1 rxn	Vol: 50 rxns	Rxn conc
ThermoScript™ Reaction Mix*	2X	25 µl	1250 µl	1 X
ROX reference dye	50X	1 µl	50 µl	1 X
ThermoScript™ Plus/ Platinum® *Taq* Enzyme Mix	50X	1 µl	50 µl	1 X
Reverse primer	10 µM	1 µl	50 µl	200 nM
Forward primer	10 µM	1 µl	50 µl	200 nM
Sterile distilled H_2O**	—	11 µl	600 µl	—
Total volume	—	40 µl	2000 µl	—

* Supplied at 2X concentration: includes 400 µM of each dNTP and 6 mM $MgSO_4$

**or use DNase/RNase Free distilled water (Cat. No. 10977-015).

2. Program the instrument with one of the following thermal cycling protocols (for cDNA synthesis, use a 30-min incubation at 50°C as a starting point):

3-Step Cycling (recommended)

cDNA synthesis:
50–70ºC, 15–30 min hold
PCR:
95ºC, 5 min hold
45 cycles of:
95ºC, 15 s
55ºC, 30 s
72ºC, 30 s

2-Step Cycling (optional)

cDNA synthesis:
50–70ºC, 15–30 min hold
PCR:
95ºC, 5 min hold
45 cycles of:
95ºC, 15 s
60-65ºC, 30-45 s

3. Add 40 µl of the Master Mix to an optical PCR tube or each well of a 96-well PCR plate.
4. Add 10 µl of sample RNA to the tube or each well of the plate. Cap or seal the tube/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/plate wells. Centrifuge briefly if needed.
6. Place reaction in the real-time PCR instrument and run the program. Collect and analyze results.

Troubleshooting

Problem	Cause	Solution
Signal in controls with no template	DNA contamination	Ensure that amplification reactions are assembled in a DNA-free environment. Use of aerosol-resistant barrier tips is recommended. Take care to avoid cross-contamination between primers or template DNA in different reactions. Run PCR product on an agarose gel in an area separate from the reaction assembly area to confirm product.
	Amplification of PCR carryover products	Analyze PCR product on an agarose gel in an area separate from the reaction assembly area. Use Platinum® Quantitative PCR SuperMix-UDG as specified in the sample protocol on page 7. Since dUTP is substituted for dTTP in the reaction cocktail, any amplified DNA will contain uracil. UDG prevents reamplification of PCR carryover products by removing uracil residues from single or double stranded DNA. dU-containing DNA that has been digested with UDG is unable to serve as template in future PCRs. UDG is inactivated at high temperature during PCR thermal cycling, thereby allowing amplification of genuine target sequence(s).
	Primer dimers	Perform melting curve analysis of the PCR product; identify dimers by lower melting point temperature. Confirm that primer designs have low penalty scores (0.0-4.0) to minimize self-annealing. Redesign primers if necessary. When redesigning primers, note that you can first try redesigning only the unlabeled primer to save the cost of the LUX™ primer.
No or low signal	Instruments setting not optimal	Confirm that the cycling parameters are correct, the quencher is set to none, and the reference dye setting is correct.
	Primer/template sequences do not match	Confirm that the sequences match.
	Primer designs are not optimal	Confirm that the primer design penalty scores are within the 0.0-4.0 range and the optimal melting temperatures have been specified. Redesign primers if necessary. When redesigning primers, note that you can first try redesigning only the unlabeled primer to save the cost of the LUX™ primer.
Poor standard curve and dynamic range	Reaction is not optimized	Reoptimize reaction conditions. Prepare primer titrations if necessary.
	Reference dye not used	Use ROX reference dye as specified.

Accessory Products

Products

The following products are available for use with LUX™ primers in real-time PCR and RT-PCR protocols:

Product	Amount	Catalog no.
Platinum® Quantitative PCR SuperMix-UDG	100 rxns	11730-017
	500 rxns	11730-025
SuperScript™ First-Strand Synthesis System for RT-PCR	50 rxns	11904-018
Platinum® Quantitative RT-PCR ThermoScript™ One-Step System	100 rxns	11731-015
	500 rxns	11731-023
Platinum® *Taq* DNA Polymerase	100 rxns	10966-018
	250 rxns	10966-026
	500 rxns	10966-034
	5,000 rxns	10966-083
Micro-to-Midi Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml	15596-026
	200 ml	15596-018
Micro-FastTrack™ 2.0 mRNA Isolation Kit	20 rxns	K1520-02
ROX Reference Dye	500 µl	12223-023
DNase I, Amplification Grade (1 U/µl)	100 U	10868-015
RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 U/µl)	5,000 U	10777-019
10 mM dNTP Mix	100 µl	18427-013
DEPC-treated water	4 x 1.25 ml	10813-012

Purchaser Notification

Limited Use Label License No. 114: LUX™ Fluorogenic Primer

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