invitrogen™
by life technologies™

EXPRESS SYBR® GreenER™ qPCR SuperMixes and Two-Step qRT-PCR Kits

Catalog nos. **11782-200/01K, 11784-200/01K, 11792-200/01K, and 11794-200/01K**

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User Manual

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Kit Contents and Storage

Kit Components and Storage

EXPRESS SYBR® GreenER™ qPCR SuperMixes and Two-Step qRT-PCR Kits are shipped on dry ice. The components in each kit are listed below.

Storage: Store all components at –20°C for long-term storage. EXPRESS qPCR SuperMixes may be stored at 4–8°C for up to one month.

EXPRESS SYBR® GreenER™ qPCR Supermix Universal	**11784-200**	**11784-01K**
EXPRESS SYBR® GreenER™ qPCR SuperMix Universal	5 ml	5 × 5 ml
ROX Reference Dye	500 µl	5 × 500 µl

EXPRESS SYBR® GreenER™ qPCR Supermix with Premixed ROX	**11794-200**	**11794-01K**
EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX	5 ml	5 × 5 ml

EXPRESS Two-Step SYBR® GreenER™ Universal	**11782-200**	**11782-01K**
EXPRESS SYBR® GreenER™ qPCR SuperMix Universal	5 ml	5 × 5 ml
ROX Reference Dye	500 µl	5 × 500 µl
SuperScript® VILO™ cDNA Synthesis Kit – 5X VILO™ Reaction Mix – 10X SuperScript® Enzyme Mix	50 rxns (20 µl each)	250 rxns (20 µl each)

EXPRESS Two-Step SYBR® GreenER™ with Premixed ROX	**11792-200**	**11792-01K**
EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX	5 ml	5 × 5 ml
SuperScript® VILO™ cDNA Synthesis Kit – 5X VILO™ Reaction Mix – 10X SuperScript® Enzyme Mix	50 rxns (20 µl each)	250 rxns (20 µl each)

Intended Use

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

Overview

Introduction

EXPRESS SYBR® GreenER™ qPCR SuperMixes and Two-Step qRT-PCR Kits provide components for real-time quantitative PCR (qPCR) and two-step reverse-transcription qPCR (qRT-PCR). Components are provided in convenient SuperMix formats and are compatible with both rapid and standard qPCR cycling conditions.

All EXPRESS SYBR® GreenER™ qPCR SuperMixes include Platinum® *Taq* DNA polymerase, SYBR® GreenER™ fluorescent dye, $MgCl_2$, heat-labile uracil DNA glycosylase (UDG), dNTPs (with dUTP instead of dTTP), and stabilizers.

- **SuperMix with Premixed ROX:** The qPCR SuperMix with premixed ROX includes ROX Reference Dye at a final concentration of 500 nM to normalize the fluorescent signal on instruments that are compatible with this option.
- **Universal SuperMix:** The Universal SuperMix includes ROX as a separate component for instruments that use ROX at a different concentration or do not require ROX.
- **Two-Step qRT-PCR Kits:** These kits include a qPCR SuperMix and the SuperScript® VILO™ cDNA Synthesis Kit for cDNA synthesis prior to qPCR. The VILO™ kit provides enhanced cDNA synthesis efficiency and can be used with very low and very high amounts of input RNA (from 1 pg up to 2.5 µg total RNA in a 20-µl reaction), giving a linear response in message abundance as measured by qPCR

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Overview, continued

Advantages of the Kits

- **Highly robust qPCR SuperMix** can accommodate a wide range of cycling conditions and reaction volumes, and combines highly sensitive detection with a broad quantification range
- **SYBR® GreenER™** dye in these formulations provides higher sensitivity and lower PCR inhibition than other fluorescent double-stranded DNA binding dyes
- **Platinum® *Taq* DNA Polymerase** provides an automatic antibody-mediated "hot start" in PCR for increased sensitivity, specificity, and yield, and has a short activation time for the rapid cycling of fast qPCR instruments
- **Heat-labile UDG and dUTP** in the SuperMix prevent amplification of carryover PCR products between reactions, and the heat-labile form of the enzyme is completely inactivated during normal qPCR cycling, eliminating any downstream degradation of amplicons
- **SuperScript® VILO™ cDNA Synthesis Kit**, included in the two-step kits, provides high yields of cDNA and linear output over a very broad range of RNA input quantities

SYBR® GreenER™ Fluorescent Dye

SYBR® GreenER™ fluorescent dye is a double-stranded DNA (dsDNA) binding dye that, in this formulation, provides higher sensitivity and lower PCR inhibition than SYBR® Green I dye. It can be used on real-time PCR instruments calibrated for SYBR® Green I dye without any change of filters or settings. In qPCR, as dsDNA accumulates, SYBR® GreenER™ dye generates a signal that is proportional to the DNA concentration (Ishiguro *et al.*, 1995; Wittwer *et al.*, 1997).

Platinum® *Taq* DNA Polymerase

Platinum® *Taq* DNA Polymerase is recombinant *Taq* DNA polymerase complexed with proprietary antibodies that block polymerase activity at ambient temperatures (Chou *et al.*, 1992; Sharkey *et al.*, 1994). Activity is restored after the initial denaturation step in PCR cycling, providing an automatic hot start in qPCR for increased sensitivity, specificity, and yield.

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Overview, continued

Heat-labile Uracil DNA Glycosylase (UDG)

Heat-labile UDG and dUTP in the qPCR SuperMix prevent the reamplification of carryover PCR products between reactions (Lindahl *et al.*, 1977; Longo *et al.*, 1990). dUTP ensures that any amplified DNA will contain uracil, while heat-labile UDG removes uracil residues from single- or double-stranded DNA.

The heat-labile form of UDG used in this kit is completely inactivated at temperatures of 50°C and higher and will not degrade amplicons following qPCR, thus enabling their use for downstream applications such as cloning.

ROX Reference Dye

ROX Reference Dye is either premixed in the qPCR SuperMix or included as a separate component to normalize the fluorescent signal between reactions for instruments that are compatible with this option.

Additional Materials Required

The following items are supplied by the user:

- Template RNA (Two-Step Kits) or DNA (qPCR SuperMixes only)
- Gene-specific primers
- DEPC-treated water
- Microcentrifuge
- Thermal cycler
- Optional: Normalization dye for instruments that do not use ROX
- PCR tubes/plates

Instrument Compatibility

Universal SuperMix

EXPRESS SYBR® GreenER™ qPCR SuperMix Universal includes ROX Reference Dye as a separate tube, and can be used with a wide range of real-time instruments, including the following:

- **Applied Biosystems:** 7900HT, 7300, 7500, StepOne™, StepOnePlus™, GeneAmp® 5700, and PRISM® 7000 and 7700
- **Bio-Rad/MJ Research:** iCycler® iQ, iQ5, and MyiQ™; DNA Engine Opticon® and Opticon® 2; and Chromo4™ Real-Time Detector
- **Cepheid:** Smart Cycler®
- **Corbett Research:** Rotor-Gene™ 3000
- **Eppendorf:** Mastercycler® ep *realplex*
- **Roche:** LightCycler® 480
- **Stratagene:** Mx3000P™, Mx3005P™, and Mx4000®

Kits with Premixed ROX

EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX can be used with real-time instruments that are compatible with ROX Reference Dye at a final concentration of 500 nM. These include the following **Applied Biosystems** instruments:

- 7900HT
- 7300
- StepOne™
- StepOnePlus™
- GeneAmp® 5700
- PRISM® 7000 and 7700

Methods

First-Strand cDNA Synthesis

Introduction

This section provides guidelines and a protocol for first-strand cDNA synthesis from RNA using the SuperScript® VILO™ cDNA Synthesis Kit, which is included with the two-step kits and is also available separately. If you are performing qPCR using DNA from another source, you can skip this section.

SuperScript® VILO™ cDNA Synthesis Kit

This cDNA synthesis kit is included with the EXPRESS Two-Step SYBR® GreenER™ qRT-PCR Kits, and is also available separately (see page 20 for ordering information). It provides enhanced cDNA synthesis efficiency and can be used with very low and very high amounts of input RNA (up to 2.5 µg total RNA in a 20-µl reaction), giving a linear response in message abundance as measured by qPCR.

The **10X SuperScript® Enzyme Mix** includes SuperScript® III RT, RNaseOUT™ Recombinant Ribonuclease Inhibitor, and a proprietary helper protein.

The **5X VILO™ Reaction Mix** includes random primers, $MgCl_2$, and dNTPs in a buffer formulation that has been optimized for qRT-PCR.

Input RNA

- Starting material using the VILO™ kit can range up to 2.5 µg total RNA in a 20-µl cDNA synthesis reaction. Note that for qPCR using SYBR® GreenER™ SuperMixes, you will need to dilute the cDNA generated from total RNA quantities above 100 ng.
- To isolate total RNA, we recommend the PureLink™ Micro-to-Midi™ Total RNA Purification System, TRIzol® Reagent, or the PureLink™ 96 Total RNA Purification Kit (see page 20). Isolation of mRNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs.
- High-quality, intact RNA is essential for accurate quantification in qRT-PCR.
- DNase I, Amplification Grade, may be used to eliminate genomic DNA contamination from the total RNA (see page 20).

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First-Strand cDNA Synthesis, continued

General Handling of RNA

When working with RNA:

- Use proper microbiological aseptic technique.
- Wear latex gloves while handling reagents, materials, and RNA samples to prevent RNase contamination.
- Use disposable, individually wrapped, sterile plasticware for all procedures.
- Use aerosol-resistant pipette tips.
- Dedicate a separate set of pipettes, buffers, and enzymes for RNA work.
- Use RNase-free microcentrifuge tubes. To decontaminate untreated tubes, soak overnight in a 0.01% (v/v) aqueous solution of diethylpyrocarbonate (DEPC), rinse with sterile distilled water, and autoclave.

RNase *Away*™ Reagent, a non-toxic solution available from Invitrogen, can be used to remove RNase contamination from surfaces.

Determining Total RNA Quality

Total RNA quality can be analyzed using a bioanalyzer such as the Agilent 2100 bioanalyzer with an RNA LabChip®. Alternatively, total RNA can be analyzed by agarose gel electrophoresis. RNA isolated using the PureLink™ kits or TRIzol® Reagent typically has a 28S-to-18S band ratio of >1.5. RNA is judged to be intact if discreet 28S and 18S ribosomal RNA bands are observed.

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Determining Total RNA Yield

Total RNA can be quantitated using the Quant-iT™ RNA Assay Kit or UV absorbance at 260 nm.

Quant-iT™ RNA Assay Kit

The Quant-iT™ RNA Assay Kit provides a rapid, sensitive, and specific method for RNA quantitation with minimal interference from DNA, protein, or other common contaminants that affect UV absorbance readings.

The kit contains a quantitation reagent and pre-diluted standards for a standard curve. The assay is performed in a microtiter plate and can be read using a standard fluorescent microplate reader.

UV Absorbance

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.5. Mix well. Transfer to a cuvette (1-cm path length).

   Note: The RNA must be in a neutral pH buffer to accurately measure the UV absorbance.

2. Determine the OD_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.

Calculate the amount of total RNA using the following formula:

Total RNA (µg) = OD_{260} × [40 µg/(1 OD_{260} × 1 ml)] × dilution factor × total sample volume (ml)

Example:

Total RNA was eluted in water in a total volume of 150 µl. A 40-µl aliquot of the eluate was diluted to 500 µl in 10 mM Tris-HCl, pH 7.5. An OD_{260} of 0.188 was obtained. The amount of RNA in the sample is:

Total RNA (µg) = 0.188 × [40 µg/(1 OD_{260} × 1 ml)] × 12.5 × 0.15 = 14.1 µg

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First-Strand cDNA Synthesis, continued

Guidelines for cDNA Synthesis

- Shorter incubation times and/or higher temperatures may be used (*e.g.*, 50°C for 30 minutes), but may result in reduced yields of cDNA.
- For increased yields of cDNA, longer incubation times may be used (up to 120 minutes at 42°C).

cDNA Synthesis Protocol

The following protocol has been optimized for generating first-strand cDNA using the SuperScript® VILO™ cDNA Synthesis Kit. The reaction volume may be scaled as needed up to 100 µl.

1. For a single reaction, combine the following components in a tube on ice. For multiple reactions, prepare a master mix without RNA.

5X VILO™ Reaction Mix	4 µl
10X SuperScript® Enzyme Mix	2 µl
RNA (up to 2.5 µg)	x µl
DEPC-treated water	to 20 µl

2. Gently mix tube contents and incubate at 25°C for 10 minutes.
3. Incubate tube at 42°C for 60 minutes.
4. Terminate the reaction at 85°C at 5 minutes.
5. Use diluted or undiluted cDNA in qPCR (see the guidelines for cDNA use in qPCR on page 9). Alternatively, store the cDNA at –20°C until use.

General qPCR Guidelines and Parameters

qPCR Setup and Conditions

- Starting material for qPCR can be cDNA, genomic DNA, or plasmid DNA
- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Make sure all equipment that comes in contact with DNA is sterile, including pipette tips and microcentrifuge tubes
- qPCR reaction volumes can be scaled from 5 µl to 100 µl, depending on the instrument.
- For instrument-specific guidelines, see the section for each type of SuperMix.

cDNA

If you are using cDNA as starting material:

- If you started with ≤100 ng of total RNA, up to 10% of the qPCR reaction volume may be undiluted cDNA (*e.g.*, for a 20-µl qPCR, use up to 2 µl of undiluted cDNA).
- If you started with >100 ng total RNA, we recommend diluting the cDNA prior to qPCR, because higher concentrations of cDNA will affect the signal baseline in SYBR GreenER™ SuperMix reactions. For example, if you started with 2 µg of total RNA, prepare a 20-fold dilution of the resulting cDNA to achieve the concentration equivalent of starting with 100 ng of RNA. Then use up to 2 µl of the diluted cDNA in a 20-µl qPCR (≤10% of qPCR volume).
- Note that detecting high-abundance genes in undiluted cDNA may result in very low CTs in qPCR, leading to reduced quantification accuracy. Prepare a dilution series of the cDNA template for the most accurate results.

Genomic or Plasmid DNA

If you are using genomic or plasmid DNA as starting material:

- Use up to 100 ng of genomic DNA or 10–10^7 copies of plasmid DNA in a 10-µl volume
- Note that 1 µg of plasmid DNA contains 9.1×10^{11} copies divided by the plasmid size in kilobases

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General qPCR Guidelines and Parameters, continued

Primer Specifications

Primer design is one of the most important parameters when using EXPRESS SYBR® GreenER™ qPCR SuperMixes. We strongly recommend using a primer design software program such as OligoPerfect™, available on the Web at www.invitrogen.com/oligos, or Vector NTI™. In addition to designing primers for optimal efficiency, programs such as these will automatically perform a BLAST search of NCBI databases to ensure that primers are target-specific.

When designing primers, the amplicon length should be approximately 80–250 bp. A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 100 and 500 nM.

Melting Curve Analysis

Melting curve analysis should always be performed following real-time qPCR to identify the presence of primer dimers and analyze the specificity of the reaction. Program your instrument for melting curve analysis using the instructions provided with your specific instrument.

Universal Kits—Guidelines and Protocols

Introduction

This section provides guidelines and protocols for using EXPRESS SYBR® GreenER™ qPCR SuperMix Universal.

Additional Materials Required

The following items are supplied by the user:

- DNA starting material
- DEPC-treated water
- Gene-specific primers
- Microcentrifuge
- Thermal cycler
- PCR tubes/plates

ROX Reference Dye Concentration

ROX Reference Dye is supplied as a separate tube in the Universal Kits. ROX is recommended for fluorescence normalization on Applied Biosystems instruments, and is optional for Stratagene and Eppendorf instruments. It is not required on other instruments.

ROX is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester and is supplied at a concentration of 25 µM.

Use the following table to determine the amount of 25-µM ROX to use with a particular instrument:

Instrument	Amount of ROX per 20-µl reaction	Effective Fold Concentration of 25-µM ROX	Final ROX Concentration
AB 7300, 7900HT, StepOne™, StepOnePlus™, and PRISM® 7000 and 7700	0.4 µl	50X	500 nM
AB 7500; Stratagene Mx3000P™, Mx3005P™, and Mx4000®	0.04 µl	500X	50 nM

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Fluorescein for Bio-Rad iCycler® Instruments

Bio-Rad iCycler® instruments require the collection of "well factors" before each run to compensate for any instrument or pipetting non-uniformity. Well factors for SYBR® GreenER™ experiments are calculated using an additional fluorophore, fluorescein. Well factors are collected using either a separate plate containing fluorescein in each well (External Well Factors) or the experimental plate with fluorescein spiked into the qPCR master mix (Dynamic Well Factors). You must select the method when you start each run using the iCycler®.

Fluorescein is available separately from Bio-Rad, or Fluorescein NIST-Traceable Standard is available from Invitrogen as a 50-µM solution (see page 20 for ordering information).

External Well Factors: The Bio-Rad iCycler® instruction manual provides instructions on preparing and using the External Well Factor plate. The iCycler® will automatically insert a 3-cycle program before your experimental cycling program to perform the External Well Factor reading.

> **Note:** The iCycler® iQ5 and MyiQ™ systems allow you to save the data from an External Well Factor reading as a separate file, which can then be referenced for future readings. Select the **Persistent Well Factor** setting when you are entering the cycling program to reference this saved file.

Dynamic Well Factors: For Dynamic Well Factor readings, the user must add fluorescein to the qPCR master mix at a final concentration of 10–20 nM. Consult your Bio-Rad iCycler® instruction manual for details.

Note that if you select the Dynamic Well Factor option, the instrument will automatically insert a 90-second incubation at 95°C before the initial 95°C denaturation step.

Continued on next page

Universal Kits, continued

General Cycling Programs

The following cycling programs have been developed as a general starting point when using EXPRESS SYBR® GreenER™ qPCR SuperMix Universal. The fast cycling program was developed using the AB 7500 in Fast mode.

Note: This mix is highly robust and can be used with a wide range of cycling programs on different instruments. If you have an alternative program that you want to use, test it with this mix.

Fast Cycling Program (developed using the AB 7500 in Fast mode)	**Standard Cycling Program**
95°C for 20 seconds 40 cycles of: 95°C for 3 seconds 60°C for 30 seconds Optional: Melting curve analysis: 60°C–95°C (refer to instrument manual for specific programming)	50°C for 2 minutes (UDG incubation) 95°C for 2 minutes 40 cycles of: 95°C for 15 seconds 60°C for 1 minute Optional: Melting curve analysis: 60°C–95°C (refer to instrument manual for specific programming)

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Roche LightCycler® 480 Cycling Program

The following cycling program is specific for the Roche LightCycler® 480 with a 96-well or 384-well plate when using EXPRESS SYBR® GreenER™ qPCR SuperMix Universal. For detailed programming instructions, consult the instrument manual.

Program Name	Cycles	Analysis Mode
Pre-incubation	1	None
Amplification	40–45	Quantification
Melting Curve	1	Melting Curves
Cooling	1	None

Target (°C)	Acquisition Mode	Hold Time (hh:mm:ss)	Ramp Rate (°C/s)	
			96-well	384-well
Pre-incubation				
95	None	00:05:00	4.4 (or 2.0)[1]	4.8
Amplification				
95	None	00:00:10	4.4 (or 2.0)[1]	4.8
Primer T_m minus 5°C [2]	None	00:00:05–00:00:20 [3]	2.2	2.5
72	Single	00:00:05–00:00:20 [3]	4.4 (or 2.0)[1]	4.8
Melting Curve				
95	None	00:00:05	2.0	2.0
65	None	00:01:00	2.0	2.0
97	Continuous (5–10 acquisitions per °C	—	—	—
Cooling				
40	None	00:00:10	2.0	2.0

[1] A ramp rate of 2.0°C/s is recommended for reaction volumes of 50 µl or greater.
[2] The annealing temperature will vary depending on the melting temperature (T_m) of the primers. Use primer T_m minus 5°C as a general starting point.
[3] Longer annealing and extension times may result in greater precision in target quantification.

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384-Well Plate Volumes

For 384-well plates, we recommend a maximum reaction volume of 10 µl per well.

qPCR Protocol

Use the protocol below as a general starting point for qPCR with EXPRESS SYBR® GreenER™ qPCR SuperMix Universal. Scale the reaction volume as needed for your real-time instrument.

ROX is recommended for Applied Biosystems instruments and optional for Stratagene and Eppendorf instruments (see page 11). Bio-Rad iCycler® instruments use fluorescein instead of ROX for Dynamic Well Factor readings (see page 12).

1. Set up reactions on ice. Volumes for a 20-µl reaction size are provided; component volumes can be scaled as needed. For 384-well plates, we recommend a maximum reaction volume of 10 µl per well. **Always prepare a master mix of common components for multiple reactions.**

	20-µl rxn
EXPRESS SYBR® GreenER™ qPCR SuperMix Universal	10 µl
10 µM forward primer (200 nM final)	0.4 µl
10 µM reverse primer (200 nM final)	0.4 µl
ROX Reference Dye (25 µM)*	0.4 µl/0.04** µl
Template DNA (see page 9)	X µl
DEPC-treated water	to 20 µl

*Consult instrument documentation. The iCycler® uses fluorescein instead of ROX for Dynamic Well Factor readings (10–20 nM final concentration; see page 12).
**See the table on page 11 for the amount/concentration of ROX to use for your specific instrument.

2. Prepare no-template control (NTC) reactions to test for DNA contamination of the enzyme/primer mixes.
3. Cap or seal each PCR tube/plate, and gently mix. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.
4. Place reactions in a real-time instrument programmed as described on the previous pages. Collect data and analyze results.
5. **Optional:** The specificity of the PCR products can be checked by agarose gel electrophoresis.

Kits with Premixed ROX—Guidelines and Protocols

Introduction

This section provides guidelines and protocols for using EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX.

Additional Materials Required

The following items are supplied by the user:

- DNA starting material
- DEPC-treated water
- Gene-specific primers
- Microcentrifuge
- Thermal cycler
- PCR tubes/plates

Premixed ROX Concentration

ROX Reference Dye is included in the SuperMix at a final concentration of 500 nM, which is compatible with Applied Biosystems 7900HT, 7300, StepOne™, StepOnePlus™, GeneAmp® 5700, and PRISM® 7000 and 7700.

Cycling Programs

The following general cycling programs have been developed as a starting point when using EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX on various instruments. The fast cycling program is designed for the AB 7900HT and StepOne™.

Note: This mix is highly robust and can be used with a wide range of cycling programs on different instruments. If you have an alternative program that you want to use, test it with this mix.

Fast Cycling Program (developed using the AB 7900HT and StepOne™)	**Standard Cycling Program**
95°C for 20 seconds 40 cycles of: 95°C for 1 second 60°C for 20 seconds Optional: Melting curve analysis: 60°C–95°C (refer to instrument manual for specific programming)	50°C for 2 minutes (UDG incubation) 95°C for 2 minutes 40 cycles of: 95°C for 15 seconds 60°C for 1 minute Optional: Melting curve analysis: 60°C–95°C (refer to instrument manual for specific programming)

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Kits with Premixed ROX, continued

384-Well Plate Volumes

For 384-well plates, we recommend a maximum reaction volume of 10 µl per well.

qPCR Protocol

Use the protocol below as a general starting point for qPCR with EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX. Scale the reaction volume as needed for your real-time instrument.

1. Set up reactions on ice. Volumes for a 20-µl reaction size are provided; component volumes can be scaled as needed. For 384-well plates, we recommend a maximum reaction volume of 10 µl per well. **Always prepare a master mix of common components for multiple reactions.**

	20-µl rxn
EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX	10 µl
10 µM forward primer (200 nM final)	0.4 µl
10 µM reverse primer (200 nM final)	0.4 µl
Template DNA (see page 9)	X µl
DEPC-treated water	to 20 µl

2. Prepare no-template control (NTC) reactions to test for DNA contamination of the enzyme/primer mixes.
3. Cap or seal each PCR tube/plate, and gently mix. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.
4. Place reactions in a real-time instrument programmed as described on the previous page. Collect data and analyze results.
5. **Optional:** The specificity of the PCR products can be checked by agarose gel electrophoresis.

Troubleshooting

Problem	Cause	Solution
Signals are present in no-template controls, and/or multiple peaks are present in the melting curve graph	Template or reagents are contaminated by nucleic acids (DNA, cDNA)	Use melting curve analysis and/or run the PCR products on a 4% agarose gel after the reaction to identify contaminants. Take standard precautions to avoid contamination when preparing your PCR reactions. Ideally, amplification reactions should be assembled in a DNA-free environment. We recommend using aerosol-resistant barrier tips.
	Primer dimers or other primer artifacts are present	Use melting curve analysis to identify primer dimers. We recommend using validated pre-designed primer sets or design primers using dedicated software programs or primer databases. Primer contamination or truncated or degraded primers can lead to artifacts. Check the purity of your primers by gel electrophoresis.
No PCR product is evident, either in the qPCR graph or on a gel	The protocol was not followed correctly	Verify that all steps have been followed and the correct reagents, dilutions, volumes, and cycling parameters have been used.
	Template contains inhibitors, nucleases, or proteases, or has otherwise been degraded.	Purify or re-purify your template.
	Primer design is suboptimal	Verify your primer selection. We recommend using validated pre-designed primers or design primers using dedicated software programs or primer databases.

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Troubleshooting, continued

Problem	Cause	Solution
PCR product is evident on a gel, but not in the qPCR graph	qPCR instrument settings are incorrect	Confirm that you are using the correct instrument settings (dye selection, reference dye, filters, and acquisition points).
	Problems with your specific qPCR instrument	See your instrument manual for tips and troubleshooting.
PCR efficiency is above 110%	Template contains inhibitors, nucleases, or proteases, or has otherwise been degraded.	Purify or re-purify your template. Inhibitors in the template may result in changes in PCR efficiency between dilutions
	Too much sample added to reactions	Decrease the concentration of cDNA; see the guidelines for cDNA concentration on page 9
	Nonspecific products may be amplified.	Use melting curve analysis if possible, and/or run the PCR products on a 4% agarose gel after the reaction to identify contaminants. Suboptimal primer design may lead to nonspecific products. Use validated pre-designed primers or design primers using dedicated software programs or primer databases.
PCR efficiency is below 90%	The PCR conditions are suboptimal	Verify that the reagents you are using have not been freeze-thawed multiple times and have not remained at room temperature for too long. Verify that the amount of primers you are using is correct.

Appendix

Additional Products

Additional Products

Related products are available separately from Invitrogen. Ordering information is provided below. For more information, visit our website at **www.invitrogen.com** or contact Technical Service (page 20).

Product	Quantity	Catalog no.
SuperScript® VILO™ cDNA Synthesis Kit	50 rxns 250 rxns	11754-050 11754-250
RNase *Away*™ Reagent	250 ml	10328-011
DNase I, Amplification Grade	100 units	18068-015
PureLink™ Micro-to-Midi™ Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml 200 ml	15596-026 15596-018
PureLink™ Genomic DNA Mini Kit	50 preps 250 preps	K1820-01 K1820-02
Quant-iT™ RNA Assay Kit	1 kit	Q-33140
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Fluorescein NIST-Traceable Standard (50 µM)	5 × 1 ml	F36915
Custom Primers	visit www.invitrogen.com/oligos	

Technical Support

On the Web

Visit the Invitrogen website at **www.invitrogen.com** for:

- Complete technical support contact information
- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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SDS

Safety Data Sheets (SDSs) are available at www.invitrogen.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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Technical Support, continued

Limited Warranty

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References

Chou, Q., Russell, M., Birch, D., Raymond, J., and Bloch, W. (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucl. Acids Res., 20*, 1717-1723

Ishiguro, T., Saitoh, J., Yawata, H., Yamagishi, H., Iwasaki, S., and Mitoma, Y. (1995) Homogeneous quantitative assay of hepatitis C virus RNA by polymerase chain reaction in the presence of a fluorescent intercalater. *Anal. Biochem., 229*, 207

Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B., and Sperens, B. (1977) DNA N-glycosidases: properties of uracil-DNA glycosidase from Escherichia coli. *J. Biol. Chem., 252*, 3286-3294

Longo, M., Berninger, M., and Hartley, J. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene, 93*, 125-128

Sharkey, D. J., Scalice, E. R., Christy, K. G., Atwood, S. M., and Daiss, J. L. (1994) Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction. *Biotechnology, 12*, 506-509

Wittwer, C. T., Herrmann, M. G., Moss, A. A., and Rasmussen, R. P. (1997) Continuous fluorescence monitoring of rapid cycle DNA amplification. *BioTechniques, 22*, 130-138

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