invitrogen™
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EXPRESS qPCR SuperMixes and Two-Step qRT-PCR Kits

Catalog nos. 11783-200/01K, 11785-200/01K, 11793-200/01K, and 11795-200/01K

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User Manual

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Kit Contents and Storage

Kit Components and Storage

EXPRESS 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 qPCR Supermix Universal	**11785-200**	**11785-01K**
EXPRESS qPCR SuperMix Universal	5 ml	5 × 5 ml
ROX Reference Dye	500 µl	5 × 500 µl

EXPRESS qPCR Supermix with Premixed ROX	**11795-200**	**11795-01K**
EXPRESS qPCR SuperMix with Premixed ROX	5 ml	5 × 5 ml

EXPRESS Two-Step Superscript® qRT-PCR Universal	**11783-200**	**11783-01K**
EXPRESS 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 Superscript® qRT-PCR with Premixed ROX	**11793-200**	**11793-01K**
EXPRESS 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)

Product Qualification

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Overview

Introduction

EXPRESS 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 qPCR SuperMixes include Platinum® *Taq* DNA polymerase, $MgCl_2$, heat-labile uracil DNA glycosylase (UDG), dNTPs (with dUTP instead of dTTP), and stabilizers. These mixes have been formulated for use with fluorogenic probe-based technology (*e.g.*, TaqMan® probes) or fluorogenic primers (*e.g.*, LUX™ Primers).

- **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

Continued on next page

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
- **Platinum® *Taq* DNA Polymerase** provides an automatic "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

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.

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.

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Overview, continued

ROX Reference Dye

ROX Reference Dye is either premixed in the 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 DNA
- Gene-specific fluorescent primers or primer/probe combinations
- DEPC-treated water
- Microcentrifuge
- Thermal cycler
- Optional: Normalization dye for instruments that do not use ROX
- PCR tubes/plates

Instrument Compatibility

Universal SuperMix

EXPRESS 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 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 SuperScript® qRT-PCR Kits, and is also available separately (see page 19 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.
- To isolate total RNA, we recommend TaqMan® Gene Expression Assays, TRIzol® Reagent, or the PureLink™ 96 Total RNA Purification Kit (see page 19). 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 19).

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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:

$$\text{Total RNA } (\mu g) = OD_{260} \times [40\ \mu g/(1\ OD_{260} \times 1\ ml)] \times \text{dilution factor} \times \text{total sample volume (ml)}$$

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:

$$\text{Total RNA } (\mu g) = 0.188 \times [40\ \mu g/(1\ OD_{260} \times 1\ ml)] \times 12.5 \times 0.15 = 14.1\ \mu g$$

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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

Reaction 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

See pages 5–8 for a cDNA synthesis protocol using the SuperScript® VILO™ cDNA Synthesis Kit. If you are using cDNA as starting material:

- Use undiluted or diluted cDNA.
- 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).
- 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

Fluorescent Primers

LUX™ Primers, available separately from Invitrogen, are a fluorescent primer-based detection technology consisting of one gene-specific primer labeled with a single fluorophore and a corresponding unlabeled primer. The labeled primer is designed with the fluorophore near the 3´ end in a hairpin structure that effectively quenches fluorescence prior to PCR, making a separate quenching moiety unnecessary. When the primer becomes incorporated into double-stranded PCR product, the fluorophore is de-quenched, resulting in a significant increase in fluorescent signal.

LUX™ Primers are available in pre-designed formats (www.invitrogen.com/lux) or can be designed for specific targets using the D-LUX™ Designer (www.invitrogen.com/dluxdesigner).

A final concentration of 200 nM per primer is effective for most reactions. Doubling the amount of reverse primer (to 400 nM) may improve the performance of certain reactions. Optimal results may require a primer titration between 100 and 500 nM.

Fluorescent Probe-Based Technologies

Fluorescent probe-based technologies, such as TaqMan® Gene Expression Assays, use two gene-specific primers with a fluorescent labeled probe that emits a signal when incorporated into the PCR product. These probe-primer combinations may be packaged as predesigned gene-specific assays or custom designed for a target of interest.

Consult the documentation provided with your fluorescent probe product to determine the optimal concentration in qPCR. EXPRESS qPCR Kits were developed using TaqMan® Gene Expression Assays provided at a 20X concentration as well as custom-designed mixtures in which the final primer concentration was 500 nM each and the final probe concentration was 200 nM.

Melting Curve Analysis

Melting curve analysis may be used with fluorescent primers to identify the presence of primer dimers and analyze the specificity of the reaction. Note that melting curve analysis cannot be used with fluorescent probe-based technologies. Program 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 qPCR SuperMix Universal.

Additional Materials Required

The following items are supplied by the user:

- DNA starting material
- DEPC-treated water
- Fluorescent primers/probes (see page 10 for information)
- 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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General Cycling Programs

The following cycling programs have been developed as a general starting point when using EXPRESS 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 *Fluorescent primers only:* 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 *Fluorescent primers only:* 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 qPCR SuperMix Universal. For detailed programming instructions, consult the instrument manual.

Program Name	Cycles	Analysis Mode
Pre-incubation	1	None
Amplification	40–45	Quantification
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	00:00:15–00:00:50 [3]	2.2 (if ≥ 50°C) [4] *or* 1.5 (if < 50°C)	2.5 (if ≥ 50°C) [4] *or* 2.0 (if < 50°C)
72	Single	00:00:01	4.4 (or 2.0)[1]	4.8
Cooling				
40	None	00:00:10	1.5	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.
[4] The ramp rate depends on the annealing temperature (see note 2 above). Use the higher ramp rate if the annealing temperature is ≥50°C.

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Universal Kits, 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 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).

1. Set up reactions on ice. Volumes for a 20-µl reaction 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 qPCR SuperMix Universal	10 µl
Fluorescent primer/probe mix (conc. and volume specified by manufacturer)	X 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

*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 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 — Kits with Premixed ROX

The following cycling programs have been developed as a general starting point when using EXPRESS qPCR SuperMix with Premixed ROX. 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. 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 *Fluorescent primers only:* 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 *Fluorescent primers only:* 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 — Kits with Premixed ROX

Use the protocol below as a general starting point for using EXPRESS 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 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 qPCR SuperMix with Premixed ROX	10 µl
Fluorescent primer/probe mix (conc. and volume specified by manufacturer)	X 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/probe sets or design them using dedicated software programs or databases. Contamination or truncated or degraded primers/probes can lead to artifacts. Check the purity of your primers and probes 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/probe design is suboptimal	We recommend using validated pre-designed primers/probes or design them using dedicated software programs or 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 sample; see the guidelines for sample 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/probes or design them 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 19).

Product	Quantity	Catalog no.
TaqMan® Gene Expression Assays	visit www.invitrogen.com/taqman	
LUX™ Fluorogenic Primers	visit www.invitrogen.com/lux	
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
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

Technical Support

Web Resources

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

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- 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).

Corporate Headquarters:
5791 Van Allen Way
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_support@invitrogen.com

Japanese Headquarters:
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail:
jpinfo@invitrogen.com

European Headquarters:
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail:
eurotech@invitrogen.com

SDS

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

Certificate of Analysis

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

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Technical Support, continued

Limited Warranty

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invitrogen™
by life technologies™

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information visit our web site at **www.invitrogen.com**