invitrogen™
by life technologies™

EXPRESS One-Step SYBR® GreenER™ Kits

For one-step qRT-PCR using EXPRESS SYBR® GreenER™ qPCR SuperMixes

Catalog nos. 11780-200, 11780-01K, 11790-200, and 11790-01K

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User Manual

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Kit Contents and Storage

Kit Components and Storage

EXPRESS One-Step SYBR® GreenER™ 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 One-Step SYBR® GreenER™ Universal	**11780-200**	**11780-01K**
EXPRESS SYBR® GreenER™ qPCR SuperMix Universal	5 ml	5 × 5 ml
ROX Reference Dye	500 µl	5 × 500 µl
EXPRESS SuperScript® Mix for One-Step SYBR® GreenER™	250 µl	5 × 250 µl

EXPRESS One-Step SYBR® GreenER™ with Premixed ROX	**11790-200**	**11790-01K**
EXPRESS SYBR® GreenER™ qPCR SuperMix with Premixed ROX	5 ml	5 × 5 ml
EXPRESS SuperScript® Mix for One-Step SYBR® GreenER™	250 µl	5 × 250 µl

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 One-Step SYBR® GreenER™ Kits provide components for one-step reverse transcription and real-time quantitative PCR (qRT-PCR) in a convenient format that is compatible with both rapid and standard qPCR cycling conditions. The one-step format allows cDNA synthesis and PCR in a single tube using gene-specific primers and either total RNA or mRNA.

The RT mix includes SuperScript® III Reverse Transcriptase and RNaseOUT™ Recombinant Ribonuclease Inhibitor in an optimized formulation. All EXPRESS SYBR® GreenER™ qPCR SuperMixes include Platinum® *Taq* DNA polymerase, SYBR® GreenER™ fluorescent dye, $MgCl_2$, uracil DNA glycosylase (UDG), dNTPs (with dUTP instead of dTTP), and stabilizers. Note that this unique one-step formulation includes a special heat-labile form of UDG in the SuperMix to help prevent reamplification of carryover PCR products between reactions.

- **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.

Continued on next page

Overview, continued

Advantages of the Kits

- This highly robust one-step formulation provides optimal convenience and sensitivity in qRT-PCR, with sensitive detection and a broad quantification range
- **SYBR® GreenER™** dye in this formulation provides higher sensitivity and lower PCR inhibition than other fluorescent double-stranded DNA binding dyes
- **SuperScript® III Reverse Transcriptase** has been engineered for reduced RNase H activity and increased thermal stability, for higher yields of cDNA
- **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
- **A special heat-labile form of UDG** in the SuperMix prevents amplification of carryover PCR products between one-step reactions

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 fluorescent signal that is proportional to the DNA concentration (Ishiguro *et al.*, 1995; Wittwer *et al.*, 1997).

SuperScript® III Reverse Transcriptase

SuperScript® III Reverse Transcriptase is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability for higher yields of cDNA (Kotewicz *et al.*, 1985). The enzyme in this RT mix formulation can synthesize cDNA at a temperature range of 50–60°C. Because SuperScript® III RT is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA.

RNaseOUT™ Ribonuclease Inhibitor is included in the SuperScript® mix to safeguard against degradation of target RNA due to ribonuclease contamination.

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Overview, continued

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)

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 UDG removes uracil residues from single- or double-stranded DNA.

The UDG used in the kit is a heat-labile form of the enzyme that destroys any contaminating dU-containing product from previous reactions prior to cDNA synthesis. This UDG is inactivated at temperatures of 50°C or higher, thereby allowing cDNA synthesis from genuine target sequences when used with a high-temperature RT such as SuperScript® III Reverse Transcriptase.

ROX Reference Dye

ROX Reference Dye is either premixed in the SuperMix or included as a separate tube in the kit 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
- 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 Kits

EXPRESS One-Step SYBR® GreenER™ 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 One-Step SYBR® GreenER™ 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

General qRT-PCR Guidelines and Parameters

Reaction Setup and Conditions

- Starting material can be total RNA or mRNA.
- These kits use a two-step cycling protocol, with a denaturation step at 95°C and an annealing/extension step at 60°C.
- Keep all components, reaction mixes and samples **on ice** to prevent premature cDNA synthesis.
- Reaction volumes can be scaled from 5 µl to 100 µl, depending on the instrument.
- For most templates, efficient cDNA synthesis can be accomplished in a 5-minute incubation at 50°C. For problematic templates, or to increase the specificity of cDNA priming, increase the cDNA synthesis temperature up to 60°C.
- For instrument-specific guidelines, see the section for each type of SuperMix.

Primer Specifications

Gene-specific primers are required for one-step qRT-PCR. We strongly recommend using a primer design 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 this will automatically perform a BLAST search of NCBI databases to ensure that primers are target-specific.

The amplicon length should be approximately 80–250 bp, and the primers should be designed to anneal to exons on both sides of an intron or within the exon/exon boundary of the target mRNA to allow differentiation of cDNA from genomic DNA.

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.

Template RNA

Input RNA

Starting material can range from 1 pg to 1 µg of purified total RNA. If you are starting with isolated mRNA, the amount of template may be as low as 0.5 pg. RNA should be free of RNase contamination and aseptic conditions should be maintained. RNA may be treated with amplification-grade DNase I (see page 19) to remove any contaminating genomic DNA.

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 for high-throughput applications (see page 19 for ordering information).

General Handling of RNA

When working with RNA:

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

You can use RNase *Away*™ Reagent, a non-toxic solution available from Invitrogen, to remove RNase contamination from surfaces. For further information on controlling RNase contamination, see (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

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Template RNA, continued

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

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.

Universal Kits—Guidelines and Protocols

Introduction

This section provides guidelines and protocols for one-step qRT-PCR using EXPRESS One-Step SYBR® GreenER™ qRT-PCR Universal.

Additional Materials Required

The following items are supplied by the user:

- DEPC-treated water
- Gene-specific primers (see page 5 for design guidelines)
- Microcentrifuge
- Thermal cycler (see page 4 for information on compatible thermal cyclers)
- 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's Mx3000P™, Mx3005P™, and Mx4000®. 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 19 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.

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Universal Kits—Guidelines and Protocols, continued

Cycling Programs — Universal Mix

The following one-step cycling programs have been developed as a general starting point when using EXPRESS One-Step SYBR® GreenER™ qRT-PCR Universal.

Program your real-time instrument to perform cDNA synthesis at or above 50°C, immediately followed by PCR amplification as shown below. The fast cycling program is designed for 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, you should test it with this mix. Note that your protocol *must* include an initial ≥50°C incubation step for UDG inactivation and cDNA synthesis.

Fast Cycling Program (for the AB 7500 in Fast mode)	**Standard Cycling Program**
50°C for 5 minutes (cDNA synthesis) 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 5 minutes (cDNA synthesis) 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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One-Step qRT-PCR — Universal Mix

Use the protocol below as a general starting point. Scale the reaction volume as needed for your real-time instrument.

ROX is recommended for Applied Biosystems® instruments and optional for Stratagene® instruments (see page 8). Bio-Rad iCycler® instruments use fluorescein instead of ROX for Dynamic Well Factor readings (see page 9).

1. Set up reactions on ice. A standard 20-µl reaction size is provided; component volumes can be scaled as desired. **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
EXPRESS SuperScript® Mix for One-Step SYBR® GreenER™	0.5 µl
Template RNA (*e.g.,* 1 pg–1 µg total RNA)	5 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 9).
**See the table on page 8 for the amount/concentration of ROX to use for your specific instrument.

2. Prepare control reactions as follows:

   No-RT controls: To test for genomic DNA contamination of the RNA sample, do not add the EXPRESS SuperScript® Mix.

   No-template controls: To test for genomic DNA contamination of the enzyme/primer mixes, do not add template RNA.

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.

Kits with Premixed ROX—Guidelines and Protocols

Introduction

This section provides guidelines and protocols for one-step qRT-PCR using EXPRESS One-Step SYBR® GreenER™ with Premixed ROX.

Additional Materials Required

The following items are supplied by the user:

- DEPC-treated water
- Gene-specific primers (see page 5 for design guidelines)
- Microcentrifuge
- Thermal cycler (see page 4 for information on compatible thermal cyclers)
- 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.

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Kits with Premixed ROX—Guidelines and Protocols, continued

Cycling Programs — Kits with Premixed ROX

The following one-step cycling programs have been developed as a general starting point when using EXPRESS One-Step SYBR® GreenER™ qRT-PCR with Premixed ROX.

Program your real-time instrument to perform cDNA synthesis at or above 50°C, immediately followed by PCR amplification as shown below. 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, you should test it with this mix. Note that your protocol *must* include an initial ≥50°C incubation step for UDG inactivation and cDNA synthesis.

Fast Cycling Program (for AB 7900HT and StepOne™)	**Standard Cycling Program**
50°C for 5 minutes (cDNA synthesis) 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 5 minutes (cDNA synthesis) 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—Guidelines and Protocols, continued

One-Step qRT-PCR — Kits with Premixed ROX

Use the protocol below as a general starting point for one-step qRT-PCR. Scale the reaction volume as needed for your real-time instrument.

1. Set up reactions on ice. A standard 20-µl reaction size is provided; component volumes can be scaled as desired. **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
EXPRESS SuperScript® Mix for One-Step SYBR® GreenER™	0.5 µl
Template RNA (*e.g.*, 1 pg–1 µg total RNA)	5 µl
DEPC-treated water	to 20 µl

2. Prepare control reactions as follows:

   No-RT controls: To test for genomic DNA contamination of the RNA sample, do not add the EXPRESS SuperScript® Mix.

   No-template controls: To test for genomic DNA contamination of the enzyme/primer mixes, do not add template RNA.

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
No PCR product is evident in the qPCR graph or on a gel	RNA has been damaged/degraded	Confirm RNA degradation by bioanalyzer or running on a gel, and replace RNA if necessary.
	RNase contamination	Maintain aseptic conditions.
	cDNA synthesis temperature too high, low priming efficiency	SuperScript® III in this formulation typically operates in a temperature range of 50–60°C.
	Primers are blocked by secondary structure	Raise the incubation temperature and/or redesign primer(s).
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).
Product detected at higher than expected cycle number	Inefficient cDNA synthesis	Adjust cDNA synthesis temperature and/or primer design.
	RT inhibitors are present in RNA	Remove inhibitors in the purified RNA by an additional 70% ethanol wash.
	RNA has been damaged/degraded	Confirm RNA degradation by Bioanalyzer or running on a gel, and replace if necessary.
	RNase contamination	Maintain aseptic conditions.
	Inefficient PCR amplification	Optimize PCR conditions by adjusting annealing temperature and/or redesigning the primers.
	Not enough template RNA	Increase concentration of template RNA to 10 ng–1 µg total RNA.
Higher than expected signal	Too much sample added to reactions	Decrease the concentration of template RNA.

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Troubleshooting, continued

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 gel after the reaction to identify contaminants. See the guidelines for avoiding contamination on page 6.
	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.

Appendix

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 on our website 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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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 17).

Product	Quantity	Catalog no.
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™ 96 Total RNA Purification Kit	4 × 96-well plates	12173-011
Quant-iT™ RNA Assay Kit	1 kit	Q-33140
Fluorescein NIST-Traceable Standard (50 µM)	5 × 1 ml	F36915
Custom Primers	visit www.invitrogen.com/oligos	

Purchaser Notification

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Purchaser Notification, continued

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