

Path-ID™ Multiplex One-Step RT-PCR Kit

TaqMan® probe-based multiplex one-step real-time RT-PCR
detection of RNA targets

Protocol

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Part Number 4425512 Rev. D
09/2010

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Safety information


Note: For general safety information, see this Preface and the “Safety” Appendix on [page 7](#). When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.


Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

 **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

 **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

 **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see “[MSDSs](#)” on [page 9](#).

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:
Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis.
For example:
Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▶) separates successive commands you select from a drop-down or shortcut menu. For example:
Select **File ▶ Open ▶ Spot Set**.
Right-click the sample row, then select **View Filter ▶ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

How to obtain support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Path-ID™ Multiplex One-Step RT-PCR Kit

Product information

Purpose of the product

The Path-ID™ Multiplex One-Step RT-PCR Kit is designed for multiplex, quantitative, reverse transcription PCR (qRT-PCR). It is optimized for the amplification of up to four animal pathogen targets simultaneously using your RNA samples and TaqMan® primer/probe sets. Reactions are run using a single-tube, one-step procedure to reverse-transcribe the RNA and amplify your targets using a Multiplex Enzyme Mix containing AmpliTaq Gold® DNA Polymerase. Reactions are run and detected in a thermal cycler capable of real-time detection of amplification products to yield quantitative data.

Figure 1 below shows amplification plots from reactions that included four targets using the Path-ID Multiplex One-Step RT-PCR Kit. Three of the targets in the experiment were held constant, but the fourth was serially diluted to show the dynamic range of multiplex target detection with the kit.

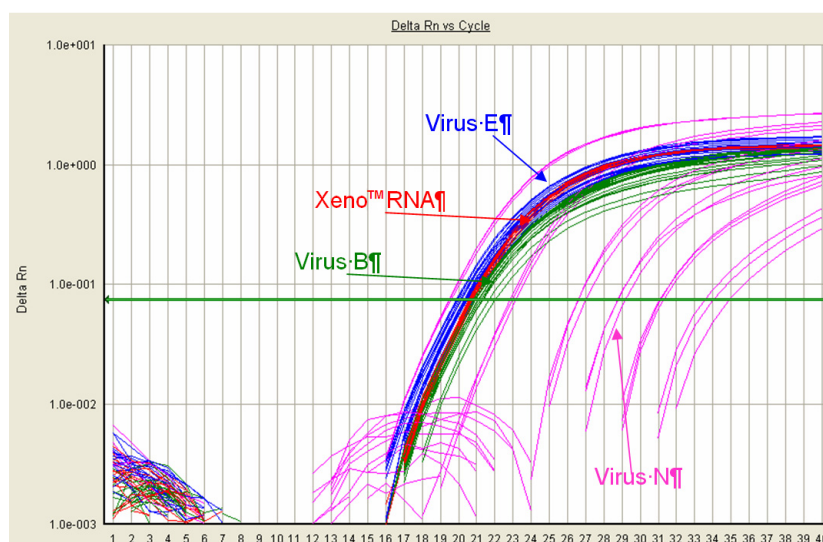


Figure 1 Four-plex amplification of control RNAs using the Path-ID™ Multiplex One-Step RT-PCR Kit

Xeno™ RNA Control and control RNAs for virus B, virus E, and virus N were amplified in a multiplex RT-PCR using the Path-ID™ Multiplex One-Step RT-PCR Kit on an Applied Biosystems 7500 Real-Time PCR System. This experiment included a sample set with fixed amounts of three of the targets and a serial dilution series of the virus N control RNA. Note that virus N was detected even in four-plex reactions containing only 400 copies (~33 C_T).

Kit contents

Component	PN 4442135 100 reactions	PN 4442136 500 reactions	PN 4442137 1000 reactions
Multiplex RT-PCR Buffer	1.375 mL	7 mL	14 mL
Multiplex Enzyme Mix	280 µL	1.4 mL	2 × 1.4 mL
Nuclease-free Water	1.75 mL	25 mL	25 mL

Storage

- Store the kit at -20 °C in a non-frost-free freezer.
- You can store the Nuclease-free Water at -20 °C, 4 °C, or at room temperature.

Materials and equipment required

RNA sample(s)

It is important to use pure RNA that is free of RT-PCR inhibitors in the procedure. We recommend the Ambion® MagMAX™ RNA Isolation Kit appropriate for your sample type; go to www.ambion.com, then search for **MagMAX**.

When isolating viral RNA from cell-free sample sources such as serum, use MagMAX™ Viral RNA Isolation Kits, which include carrier RNA to maximize viral RNA recovery.

PCR primer/TaqMan® probe mixture

You can use any licensed PCR primer/TaqMan probe mixture compatible with your real-time PCR instrument and designed for one-step RT-PCR with the kit. Optimization of PCR primer and probe concentrations is critical for multiplex reactions. In reactions with targets of different abundance, it is necessary to limit the PCR primer concentrations of highly abundant targets so that less abundant targets can effectively compete for the amplification reagents. The concentration of primers and probes may require optimization, but the concentrations shown in the table below typically work well.

Component	Final concentration in the reaction	25× primer/probe mix [‡]
Forward PCR primer	400 nM	10 μM
Reverse PCR primer	400 nM	10 μM
TaqMan® probe	120 nM	3 μM

[‡] Use 1 μL per 25-μL RT-PCR of a PCR primer/TaqMan probe mixture prepared at these concentrations.

Real-time PCR systems and accessories

The Path-ID™ Multiplex One-Step RT-PCR Kit is compatible with the following Applied Biosystems systems:

- 7500 Real-Time PCR System
- 7500 Fast Real-Time PCR System
- 7900HT Real-Time PCR System (96-well and 384-well sample block)
- 7900HT Fast Real-Time PCR System (96-well and 384-well sample block)
- StepOne™ Real-Time PCR System
- StepOnePlus™ Real-Time PCR System

You need the following accessories:

- Reaction plates and covers appropriate for your real-time PCR system. See the Plastic Consumables Compatibility Chart: go to www.appliedbiosystems.com, then select **Products ▶ Real-Time PCR ▶ Reaction Plates & Adhesive Films**.
- Nuclease-free pipettes and tips.
- Reagent reservoirs or tubes for preparing the master mixes.

Procedure

For the following hazards, see the complete safety alert descriptions in “[Chemical alerts](#)” on page 10.



CAUTION! CHEMICAL HAZARD. Multiplex RT-PCR Buffer.



CAUTION! CHEMICAL HAZARD. Multiplex Enzyme Mix.

Program the real-time PCR instrument

Use the thermal cycling conditions shown in the table below.

- ROX™ passive reference dye is included in the Multiplex RT-PCR Buffer.
- For real-time PCR instruments capable of Fast thermal cycling, set the mode to *Standard*.
- The suggested reaction volume is 25 µL.

	Stage	Reps	Temp	Time
Reverse transcription	1	1	48 °C	10 min
RT inactivation/ initial denaturation	2	1	95 °C	10 min
Amplification	3	40	95 °C	15 sec
			60 °C	45 sec [‡]

[‡] For long targets, the extension time may need to be >45 seconds.

Prepare the reactions

1. Prepare the RT-PCR master mix(es) on ice (see the required volumes in the table below):
 - Prepare 5 to 10% extra master mix.
 - Include duplicate no template controls (NTCs or negative controls) using Nuclease-free Water in place of sample.
2. Add the RT-PCR master mix to a reaction plate or tubes.
3. Add sample to each reaction.

	Component	Volume (µL)
RT-PCR master mix	Multiplex RT-PCR Buffer	12.5
	Forward and reverse PCR primers	—
	TaqMan® probes	—
	Multiplex Enzyme Mix	2.5
	RNA sample (Nuclease-free Water for NTCs)	—
	Total volume per reaction	25.0

Perform thermal cycling and analyze the data

Follow the instructions for your real-time PCR instrument for multiplex reactions.

Troubleshooting

Observation	Possible Cause	Solution
No signal from samples expected to be positive	RNA sample contains PCR inhibitors	<ul style="list-style-type: none"> • Use less starting sample as input for your RNA isolation procedure. • Increase the number or stringency of washes during RNA isolation. • Use less RNA sample in the qRT-PCR. Follow the guidelines below.
Low signal from samples expected to be positive	RNA sample contains low level of PCR inhibitors	<p>Samples containing minimal amounts of inhibitors may yield successful qRT-PCR reactions if less RNA sample (and therefore less inhibitor) is added to the reaction. For example:</p> <ul style="list-style-type: none"> • Reduce the sample volume to 1 to 2 μL, then add Nuclease-free Water to bring the reaction to the proper volume. -Or- • Dilute the RNA sample 1:10 using the solution used to elute the nucleic acid at the end of the nucleic acid isolation procedure, then use the diluted RNA in the qRT-PCR reaction. -Or- • Dilute the RNA sample 1:10 using 10 mM Tris-HCl pH 8, 0.1 mM EDTA, then use the diluted RNA in the qRT-PCR reaction.
Signal detected in no template control (NTC)	PCR contamination	<ul style="list-style-type: none"> • Repeat the qRT-PCR reaction with fresh reagents and decontaminated pipettors. • Set up and run the qRT-PCR reaction in an area that is isolated from areas used for nucleic acid isolation and PCR product analysis.

This appendix covers:

- General chemical safety 8
- MSDSs 9
- Chemical alerts 10



General chemical safety

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See [“About MSDSs” on page 9.](#))
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to www.appliedbiosystems.com, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
 - **Open** – To view the document
 - **Print Target** – To print the document
 - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.



Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see [“Safety alert words” on page v](#).

Specific chemical alerts



CAUTION! CHEMICAL HAZARD. Multiplex RT-PCR Buffer may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



CAUTION! CHEMICAL HAZARD. Multiplex Enzyme Mix may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Part Number 4425512 Rev. D 09/2010



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