USER GUIDE

applied biosystems by life technologies™

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

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

Catalog Numbers 4428206, 4428207, 4440022

Publication Part Number 4440907 Rev. C
Revision Date May 2012

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

life technologies™

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

COPYRIGHT

DISCLAIMERS

TRADEMARKS

Contents

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

Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

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 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. The kit contains a Multiplex Enzyme Mix with ArrayScript™ reverse transcriptase and AmpliTaq Gold® DNA Polymerase, and a buffer mix. Reactions are run and detected in a thermal cycler capable of real-time detection of amplification products to yield quantitative data.

Figure 1 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.

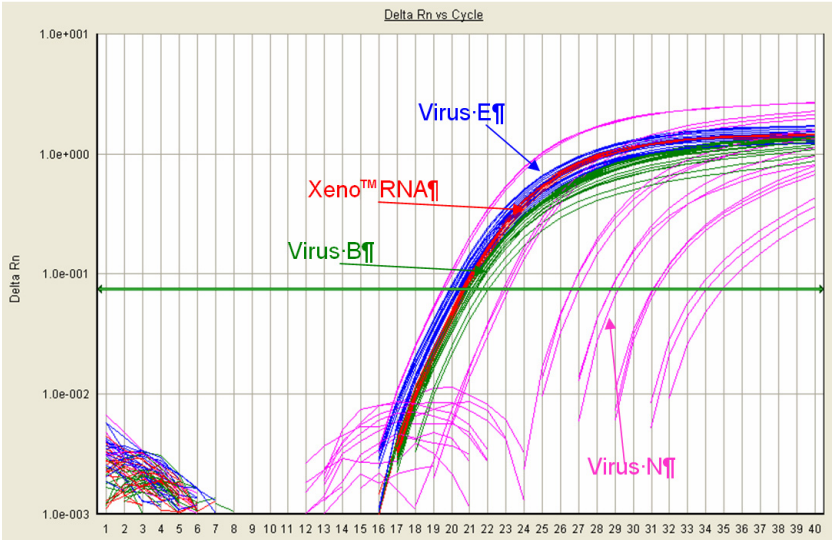

Figure 1 Four-plex amplification of control RNAs using the Path-ID™ Multiplex One-Step RT-PCR Kit

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	Cat. no. 4428206 100 reactions	Cat. no. 4428207 500 reactions	Cat. no. 4440022 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 –10°C to –30°C in a non-frost-free freezer.
- You can store the Nuclease-free Water at –10°C to –30°C, 2°C to 8°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 MagMAX™ RNA Isolation Kit appropriate for your sample type; go to **www.lifetechnologies.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 following table typically work well.

Note: The Reverse Transcriptase enzyme contained in this kit is produced using an *E. coli* expression vector containing a proprietary version of the MMLV *pol* gene (GenBank accession no. J02255) expressed from pET-24(+). It is possible that a minimal amount of the expression vector could be carried over into the final mastermix formulation. If you are targeting MMLV, a related virus, or any of the plasmid sequence, we recommend designing primer sequences not contained in the expression vector.

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 ® 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.lifetechnologies.com**, then select **Products and Services ▸ Real-Time PCR ▸ PCR tubes, Plates & Accessories**.
- 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 safety" on page 12.

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 following table.

- 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.

Step	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 following table):
   - Prepare 5–10% 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	• 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. See the solution to "Low signal from samples expected to be positive".
Low signal from samples expected to be positive	RNA sample contains low level of PCR inhibitors	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: • 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	• 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. **Note:** The Reverse Transcriptase enzyme contained in this kit is produced using an *E. coli* expression vector containing a proprietary version of the MMLV *pol* gene (GenBank accession no. J02255) expressed from pET-24(+). It is possible that a minimal amount of the expression vector could be carried over into the final mastermix formulation. If you are targeting MMLV, a related virus, or any of the plasmid sequence, we recommend designing primer sequences not contained in the expression vector.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply

Chemical alerts

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.

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**

Note: For the SDSs of chemicals not distributed by Life Technologies Corporation, contact the chemical manufacturer.

Obtaining Certificates 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.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

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

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies Corporations' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies Corporation at **www.lifetechnologies.com/support**.

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™