Multiplex Real-Time RT-PCR Analysis of Animal Pathogen RNA

Sensitive, Multiplex Amplification in a One-Step, Single-Tube Reaction

- Efficient—save time and money by amplifying up to 4 different RNA targets simultaneously
- Simple—single-tube reaction minimizes cross-contamination
- Sensitive—optimized for low-copy number amplification of RNA targets
- Robust—efficient amplification over 7 logs of RNA input amount

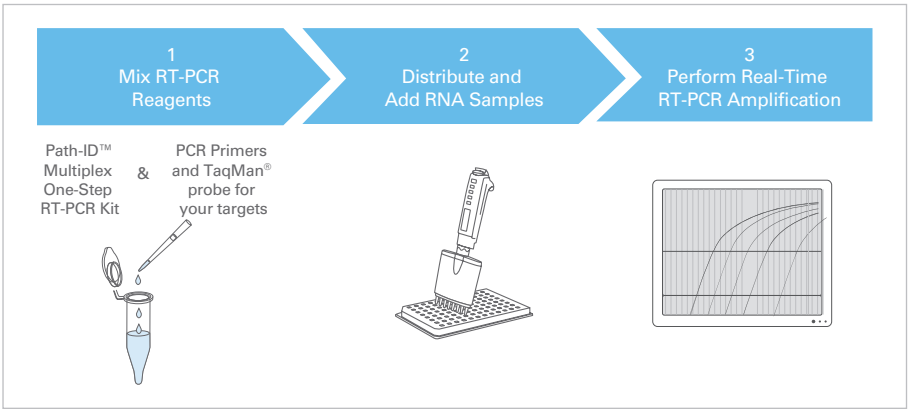

Figure 1. Path-ID™ Multiplex One-Step RT-PCR Kit Procedure Overview.

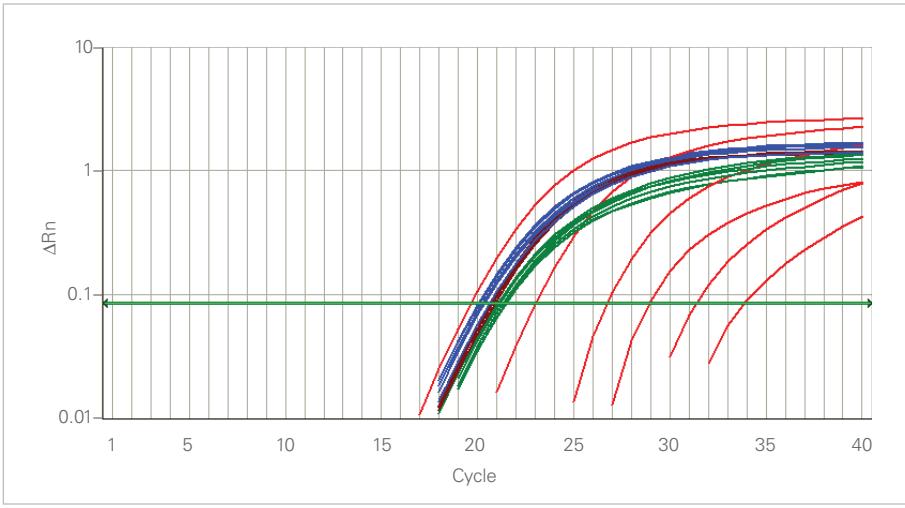

Figure 2. Path-ID™ Multiplex One-Step RT-PCR Kit Consistently Amplifies 4 Animal Pathogen RNA Targets in a Single Reaction. Xeno™ RNA Control and control RNAs for Virus A, Virus B, and Virus C were amplified in a single multiplex reaction using the Path-ID™ Multiplex One-Step RT-PCR Kit and run on the Applied Biosystems 7500. A sample set with fixed amounts of 3 of the targets and a serial dilution series of the Virus B Control RNA (red curve) were included.

The Path-ID™ Multiplex One-Step RT-PCR Kit is designed for the sensitive, robust amplification and multiplex quantitation of up to 4 RNA targets simultaneously using a simple real-time RT-PCR strategy (Figure 1). The kit includes the buffers and enzymes needed for RT-PCR; you supply only the RNA sample, PCR primers, and TaqMan® probes.

Simple, Robust Reactions

Path-ID™ Multiplex Kit reactions are run using a single-tube, one-step procedure to reverse transcribe the RNA and amplify your targets (Figure 1). Single-tube reactions minimize sample handling errors producing more consistent results. Reactions are driven with Multiplex Enzyme Mix, which includes ArrayScript™ Reverse Transcriptase, an MMLV RT capable of producing high cDNA yields. The mix also includes AmpliTaq Gold® DNA Polymerase—the preferred hot start DNA polymerase for specific target amplification, and RT-PCR Buffer with optimized reagents for efficient, robust results from both the reverse transcription reaction and the PCR. The buffer also contains ROX™ passive reference dye for quantitative fluorescent signal normalization.

Increase Efficiency with Multiplexing

The Path-ID™ Multiplex Kit is designed for the amplification of up to 4 RNA animal pathogen targets simultaneously, resulting in both time and cost savings. Figure 2

shows the amplification of 4 targets by multiplex RT-PCR using the kit. The quantities of three of the targets in the experiment were held constant, but the fourth target was serially diluted to show the dynamic range of multiplex target amplification with the kit. Furthermore, Figure 3 shows that similar sensitivity is obtained in multiplexed reactions compared to singleplex reactions.

Better Sensitivity

In comparison to a competitor's product, the Path-ID™ Multiplex Kit shows superior sensitivity with more consistent results (Figure 4).

Streamline Your Research with the Path-ID™ Multiplex One-Step RT-PCR Kit

The Path-ID™ Kit brings the sensitivity, specificity, and wide dynamic range of real-time PCR to amplification of nucleic acid target sequences. The kit is configured to amplify up to four RNA targets in a single RT-PCR, without compromising results. With a single-tube procedure, potential sample handling errors are minimized and reaction setup is fast and easy. The kit has been shown to amplify as few as 20 copies of target, providing unsurpassed sensitivity in a streamlined workflow.

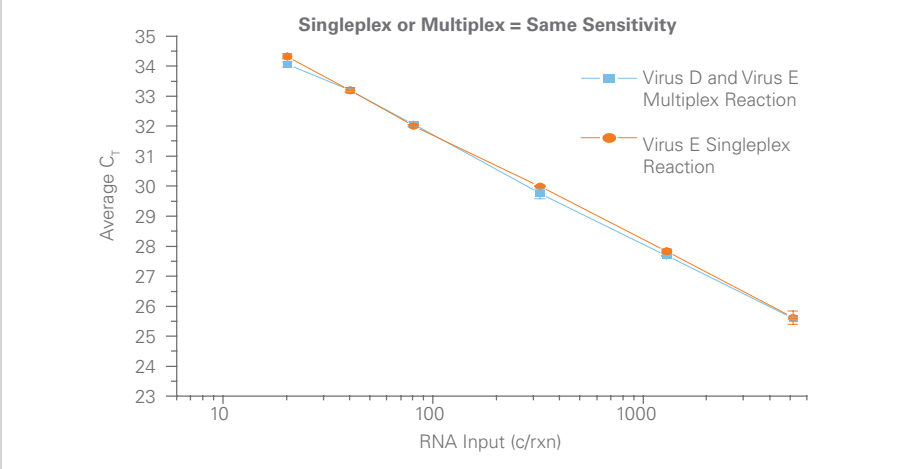

Figure 3. Path-ID™ Multiplex One-Step RT-PCR Kit Comparably Amplifies Targets in Singleplex and Duplex RT-PCRs. Virus E RNA was reverse transcribed and PCR amplified in a singleplex reaction, and Virus D and Virus E were reverse transcribed and co-amplified in a duplex reaction using the Path-ID™ Multiplex One-Step RT-PCR Kit. No difference in sensitivity was observed between the singleplex and multiplex reactions.

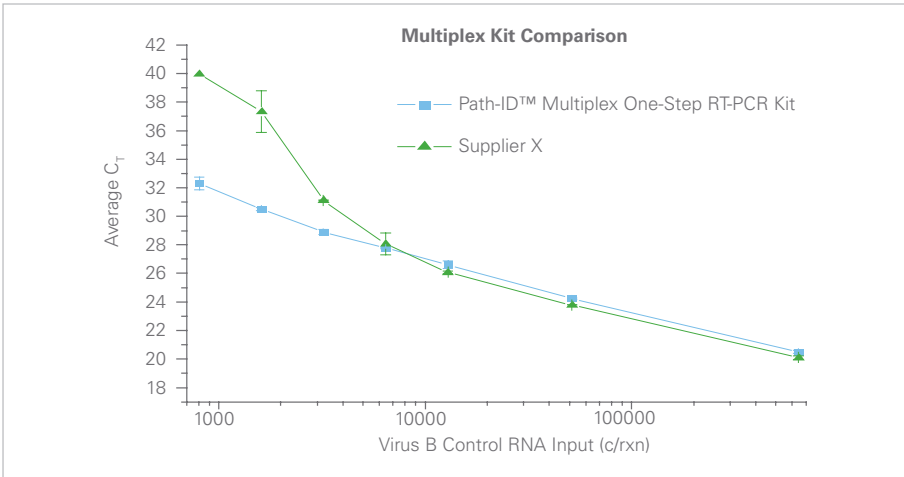

Figure 4. Path-ID™ Multiplex One-Step RT-PCR Kit Provides Higher Target Analytical Sensitivity Than a Competitor's Kit. The quadraplex RT-PCR experiment described in Figure 2 was performed using the Path-ID™ Multiplex One-Step RT-PCR Kit and a kit from Supplier X. Only data for the Virus B target are shown. The Path-ID™ Multiplex One-Step RT-PCR Kit amplified the lower amounts of target with better sensitivity (lower C_T values) than the kit from Supplier X. Note that at <1000 copies/rxn, only the Path-ID™ Multiplex One-Step RT-PCR Kit was able to amplify Virus B RNA.

ORDERING INFORMATION

Description	Quantity	Part Number
Path-ID™ Multiplex One-Step RT-PCR Kit	100 rxns	4388639
Path-ID™ Multiplex One-Step RT-PCR Kit	500 rxns	4388641
Path-ID™ Multiplex One-Step RT-PCR Kit	1000 rxns	4388642

Printed in the U.S.A. 06/2011 Publication 128PB08-02

Applied Biosystems

Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.327.3002
www.appliedbiosystems.com

International Sales
For our office locations please call the division headquarters or refer to our website at
www.appliedbiosystems.com/about/offices.cfm