



Master mixes built for the specific needs of veterinary labs

Introduction

Thermo Fisher Scientific recognizes that the value of your PCR testing results is only as good as the reagents you rely on. That's why our enzymes have been optimized to help you identify the animal pathogen targets most important to you. We have rigorously developed reagents that are robust and consistent, with the ability to perform in the presence of PCR inhibitors found in even the most challenging animal samples. Whether your lab's needs are simple or complex, or whether you are new to PCR testing or have been designing veterinary assays for years, our easy-to-use master mixes can help you feel confident in your results (Figure 1).

- Our offering includes:**
- One-step RT-PCR master mix
 - Multiplex one-step RT-PCR master mix
 - qPCR master mix
 - Master mixes with internal positive control (IPC)

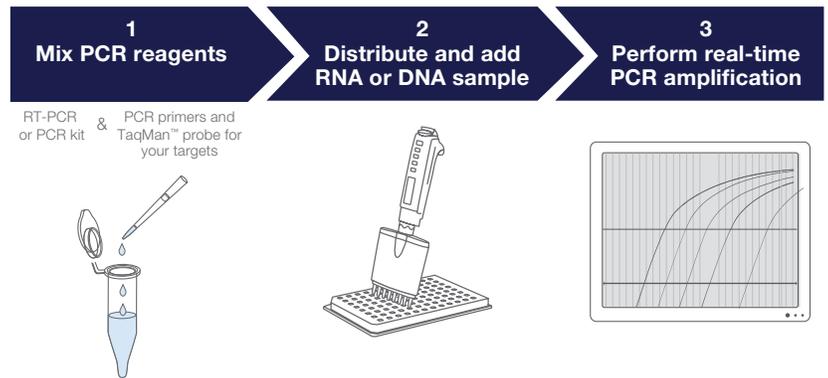


Figure 1. Workflow for master mix products from Thermo Fisher Scientific.

One-step RT-PCR master mix

Applied Biosystems™ AgPath-ID™ One-Step RT-PCR Kit—economical, high-quality, ready-to-use master mix for amplification of RNA targets.

- Consistent, reliable amplification helps provide results you can trust
- Simple, single-tube, one-step reaction minimizes handling and helps reduce the risk of cross-contamination
- A detection enhancer is provided as an optional reagent for amplification of difficult templates

Formulation

The AgPath-ID One-Step RT-PCR Kit is designed for sensitive, robust amplification of RNA targets in the presence of PCR inhibitors typically found in animal samples. The kit includes:

- 25X RT-PCR Enzyme Mix containing:
 - Invitrogen™ ArrayScript™ Reverse Transcriptase (RT), a mutant M-MLV RT that produces high cDNA yields
 - Ultra-pure, hot-start DNA polymerase providing superior specificity and sensitivity
- Optimized 2X RT-PCR Buffer for efficient, robust reverse transcription and PCR
 - Includes Invitrogen™ ROX™ dye as an internal reference for normalization and precise data analysis
- A detection enhancer as an optional reagent for amplification of templates with high GC content or persistent secondary structure

Sensitive, reliable performance

To illustrate the consistent performance of the AgPath-ID One-Step RT-PCR Kit, serial dilutions of virus A control RNA containing 5 to 5×10^6 copies were amplified (Figure 2). The amplification plot shows a consistent set of curves expected from highly efficient PCR, and the graph shows the reliability and efficiency of the reaction across a wide range of input template amounts.

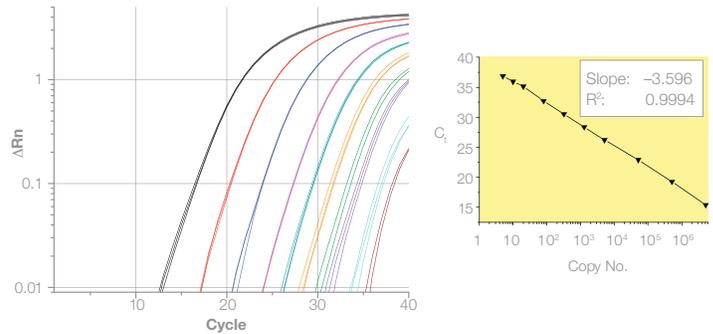


Figure 2. qRT-PCR targeting serially diluted virus A control RNA transcript (5 to 5×10^6 copies) demonstrates highly efficient and consistent performance of AgPath-ID One-Step RT-PCR Kit.

Reactions were performed on an Applied Biosystems™ 7500 Fast Real-Time PCR System.

Figure 3 shows amplification of a serial dilution of a different control RNA, virus B. Amounts of RNA were kept low (20 to 40,000 copies) in order to compare the analytical sensitivity of target amplification of the AgPath-ID kit and a competitor's RT-PCR kit. The AgPath-ID One-Step RT-PCR Kit provided earlier C_t values and better analytical sensitivity than the competitor's kit across the dilution range.

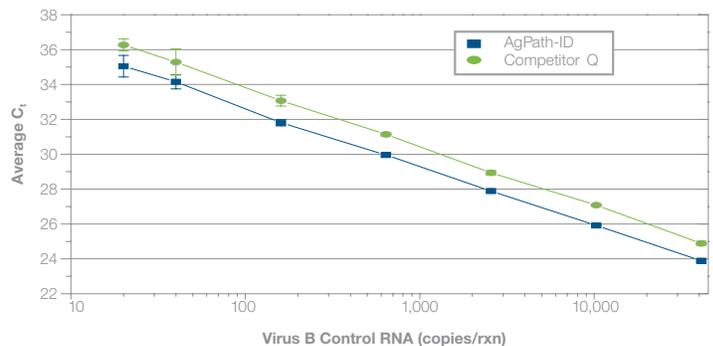


Figure 3. AgPath-ID One-Step RT-PCR Kit is more sensitive than a leading competitor's kit. Serially diluted virus B control RNA (20 to 40,000 copies) was amplified using the AgPath-ID One-Step RT-PCR Kit and a leading competitor's kit. Reactions were performed on an Applied Biosystems 7500 Fast Real-Time PCR System.

Multiplex one-step RT-PCR master mix

Applied Biosystems™ Path-ID™ Multiplex One-Step RT-PCR Kit—highly sensitive and convenient master mix optimized for veterinary labs targeting RNA pathogens.

- Multiplex amplification of up to 4 different targets simultaneously helps save time and money
- Optimized to amplify low copy (20 copies) number targets to deliver results even with challenging samples
- Capable of amplification of over 7 logs of input to provide robust performance when you need it

Formulation

The Path-ID Multiplex One-Step RT-PCR Kit is designed for the sensitive, robust amplification and multiplex quantitation of animal pathogen RNA in a simple format. The kit includes:

- Multiplex Enzyme Mix containing:
 - An M-MLV RT capable of producing high cDNA yields
 - Ultra-pure, hot-start DNA polymerase providing superior specificity and sensitivity
- Multiplex RT-PCR Buffer with optimized reagents for efficient, robust results from both the reverse transcription reaction and the PCR
 - Includes ROX dye as an internal reference for normalization and precise data analysis

Multiplex with confidence

In the study depicted in Figure 4, the Path-ID Multiplex One-Step RT-PCR Kit provides higher target analytical sensitivity in comparison to a competitor's product.

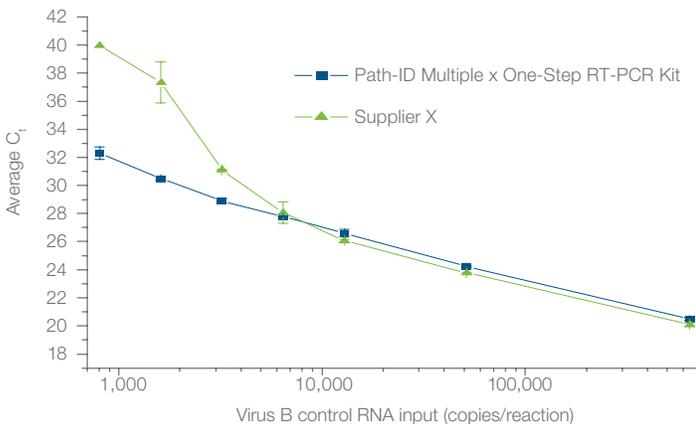


Figure 4. The Path-ID Multiplex One-Step RT-PCR Kit amplifies the lower amounts of target with better sensitivity (lower C_t values) than the competitor kit. A quadruplex RT-PCR experiment was performed using the Path-ID Multiplex One-Step RT-PCR Kit and a competitor kit. Only data for the virus B target are shown. Note that at <1,000 copies/reaction, only the Path-ID Multiplex One-Step RT-PCR Kit was able to amplify virus B RNA.

Figure 5 shows that the Path-ID Multiplex One-Step RT-PCR Kit comparably amplifies targets in singleplex and duplex RT-PCR reactions, suggesting that there is no loss of sensitivity as a result of multiplexed reactions.

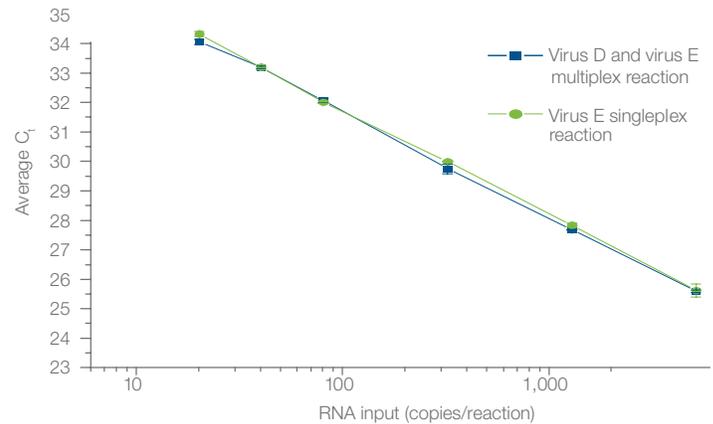


Figure 5. Path-ID Multiplex One-Step RT-PCR Kit shows no difference in sensitivity between singleplex and multiplex reactions. Virus E RNA was reverse-transcribed and PCR-amplified in a singleplex reaction, and virus D RNA and virus E RNA were reverse-transcribed and coamplified in a duplex reaction using the Path-ID Multiplex One-Step RT-PCR Kit.

Figure 6 shows the amplification of 4 targets by multiplex RT-PCR using the Path-ID Multiplex One-Step RT-PCR Kit. The quantities of 3 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. The results show that the Path-ID Multiplex One-Step RT-PCR Kit consistently amplifies 4 animal pathogen RNA targets in a single reaction.

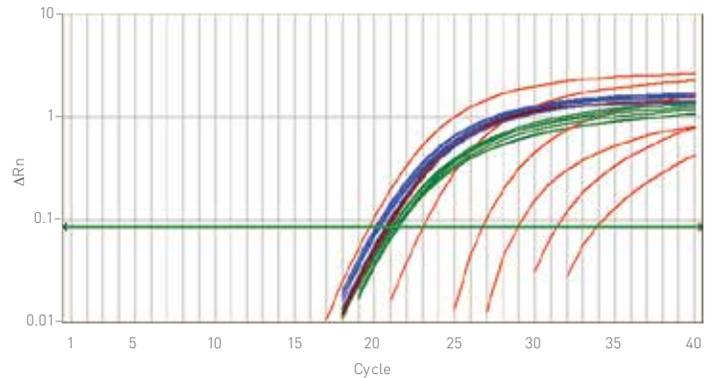


Figure 6. Path-ID Multiplex One-Step RT-PCR Kit consistently amplifies multiple pathogen targets in a single reaction. Applied Biosystems™ Xeno™ RNA Control and control RNAs for virus A, virus B, and virus C were amplified in a single multiplex reaction using the Path-ID Multiplex One-Step RT-PCR Kit and run on the Applied Biosystems 7500 Real-Time PCR System. 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.

qPCR master mix

Applied Biosystems™ Path-ID™ qPCR Master Mix—highly sensitive master mix used to detect animal pathogen DNA, optimized to perform in the presence of challenging qPCR inhibitors.

- Capable of amplifying over 7 logs of input and down to 25 copies of target for dependable, robust performance
- Inhibitor tolerance to help deliver accurate results even with challenging samples
- Stable performance at a wide temperature range allows for convenient reaction setup and reagent storage

Formulation

Path-ID qPCR Master Mix is designed for the sensitive, robust amplification of animal pathogen DNA in a convenient format. It includes:

- Ultra-pure, hot-start DNA polymerase enabling room temperature reaction setup and minimizes nonspecific PCR products
- Optimized buffer and dNTPs for enhanced sensitivity and functionality in the presence of PCR inhibitors
- ROX dye as an internal reference for normalization and precise data analysis

Convenience and performance

Figure 7 shows that Path-ID qPCR Master Mix provides dependable target amplification by producing a linear dynamic range across 7 orders of magnitude, down to 25 copies of target. Path-ID qPCR Master Mix enables amplification of even the most dilute samples.

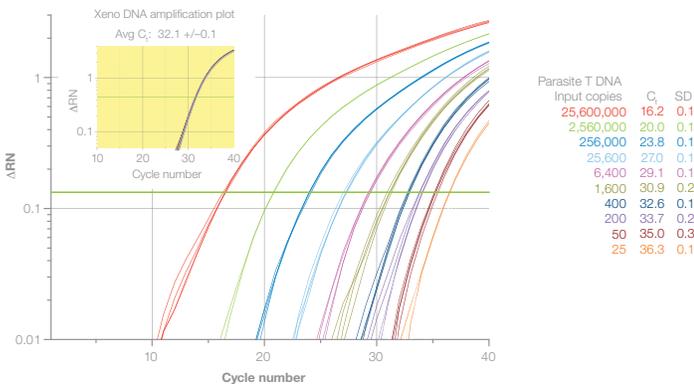


Figure 7. Amplification plot for parasite T DNA in 4 replicate reactions using Path-ID qPCR Master Mix on the Applied Biosystems 7500 Real-Time PCR System. A dilution series of parasite T DNA amplified with Path-ID qPCR Master Mix demonstrates that even the most dilute samples containing as few as 25 copies of target are easily amplified. All reactions showed consistent amplification of Xeno™ DNA Control, an internal positive control (inset).

Path-ID qPCR master mix provides reliable amplification of numerous animal pathogen DNA targets in the presence of PCR inhibitors frequently associated with agricultural samples. Figure 8 shows the ability of Path-ID qPCR Master Mix to tolerate high levels of both hematin (20 μM) and humic acid (15 ng/μL) compared to a competitor's master mix.

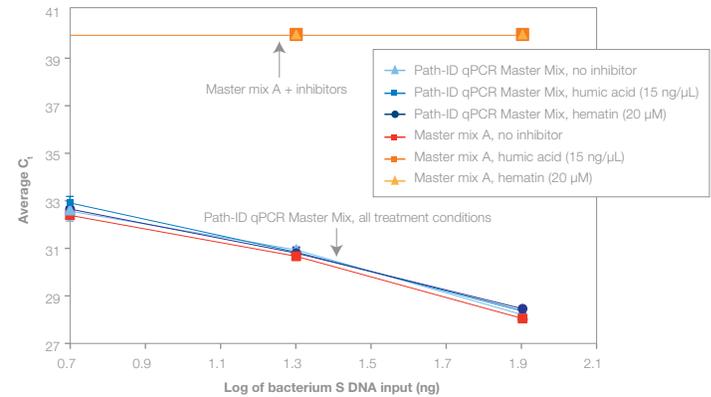


Figure 8. Path-ID qPCR Master Mix shows better tolerance to inhibitors than the competitor's master mix. C_t values are shown for amplification of a dilution series of bacterium S target DNA in the presence of the common PCR inhibitors hematin (20 μM) and humic acid (15 ng/μL). The limit of detection for C_t is set at 40; measurements ≥40 represent undetermined data.

Path-ID qPCR Master Mix retains high performance even after exposure to harsh conditions. In Figure 9, Path-ID qPCR Master Mix was subjected to multiple freeze/thaw cycles as well as room temperature treatment. In all cases, Path-ID qPCR Master Mix demonstrates equivalent amplification, exhibiting its stability during harsh storage events and even room temperature reaction setup.

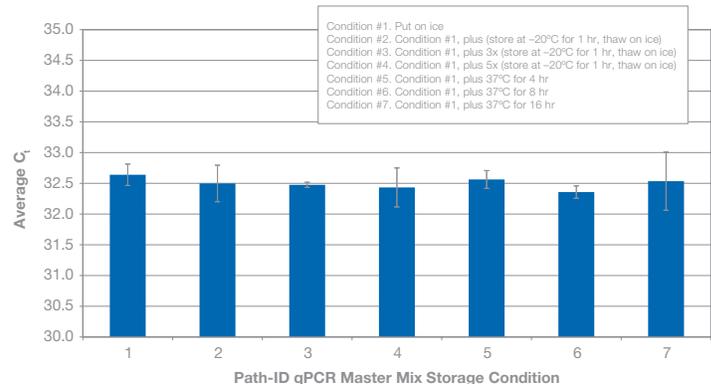


Figure 9. C_t values are given for amplification of bacterium M DNA using Path-ID qPCR Master Mix with various handling conditions. PCR was performed on bacterium M DNA using Path-ID qPCR Master Mix that had been subjected to various freeze/thaw cycles and stored at 37°C for different lengths of time. Reactions were carried out on the Applied Biosystems 7500 Real-Time PCR System. Consistent C_t values were obtained after subjecting Path-ID qPCR Master Mix to the different conditions.

Master mixes with internal positive control

Applied Biosystems™ VetMAX™-Plus master mixes provide the highly sensitive and robust performance you need with the added confidence and convenience of an included Xeno™ internal positive control (IPC). The use of an IPC in pathogen detection workflows allows you to distinguish true target negatives from PCR inhibition.

- Xeno IPC monitors the reaction for inhibition and effectiveness of nucleic acid purification, enabling greater confidence in results
- Formulations are optimized for use in detecting challenging animal RNA or DNA pathogens
- A suite of master mix options (RT-PCR, multiplex, qPCR) are available to fit your unique application

Formulations

Components of each Applied Biosystems™ VetMAX™-Plus kit are provided below.

Applied Biosystems™ VetMAX™-Plus One-Step RT-PCR Kit

- 25X RT-PCR Enzyme Mix containing:
 - ArrayScript Reverse Transcriptase, a mutant M-MLV RT that produces high cDNA yields
 - Ultra-pure, hot-start DNA polymerase providing superior specificity and sensitivity
- 2X RT-PCR Buffer for efficient, robust reverse transcription and PCR
 - Includes ROX dye as an internal reference for normalization and precise data analysis
- Xeno RNA Control

Applied Biosystems™ VetMAX™-Plus Multiplex One Step RT-PCR Kit

- 10X Multiplex Enzyme Mix containing:
 - An M-MLV RT capable of producing high cDNA yields
 - Ultra-pure, hot-start DNA polymerase providing superior specificity and sensitivity
- 2X Multiplex RT-PCR buffer for efficient, robust reverse transcription and PCR
 - Includes ROX dye as an internal reference for normalization and precise data analysis
- Xeno RNA Control

Applied Biosystems™ VetMAX™-Plus qPCR Master Mix

- 2X qPCR master mix containing:
 - Ultra-pure, hot-start DNA polymerase enables room temperature reaction setup and minimizes nonspecific PCR products
 - Optimized buffer and dNTPs for enhanced sensitivity and functionality in the presence of PCR inhibitors
 - ROX dye as an internal reference for normalization and precise data analysis
- Xeno DNA Control

Qualified results

Using Xeno IPC effectively monitors for PCR inhibition, which means that you can easily qualify your testing results. Figure 10 shows how Xeno IPC identifies the presence of a PCR inhibitor (hematin) at multiple concentrations. Since the expected range of Xeno IPC C_t values in a normal reaction (without inhibition) is known, you can determine the effect that inhibition has on the reaction, thereby lowering the risk of false negative results.

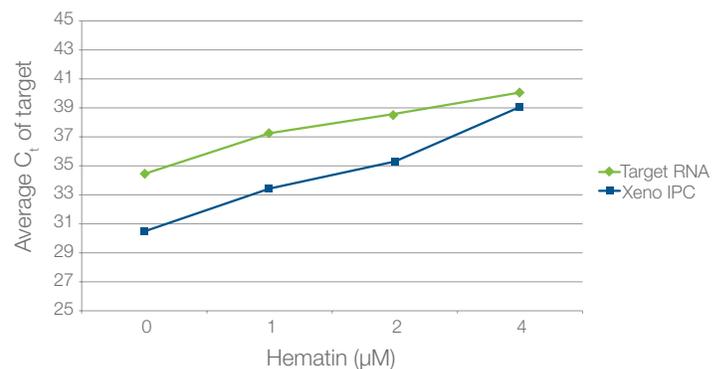


Figure 10. Graph depicting the effect of increasing inhibition on RNA target and subsequent effect on Xeno IPC. 100 copies per reaction of RNA target and 1,000 copies per reaction of Xeno IPC were exposed to increasing levels of hematin (0–4 µM). The data show that Xeno IPC follows the target's trend of increasing C_t values due to inhibition and therefore can be used as an indicator of inhibition in the reaction. Reactions were carried out on the Applied Biosystems 7500 Real-Time PCR System, run in standard mode.

For greater quality and consistency of animal RNA and DNA pathogen detection, use VetMAX-Plus master mixes with VetMAX™ reagents and controls.

Ordering information

Product	Quantity	Cat. No.
Path-ID qPCR Master Mix	100 reactions	4388643
Path-ID qPCR Master Mix	500 reactions	4388644
AgPath-ID One-Step RT-PCR Kit	100 reactions	AM1005
AgPath-ID One-Step RT-PCR Kit	500 reactions	4387424
AgPath-ID One-Step RT-PCR Kit	1,000 reactions	4387391
Path-ID Multiplex One-Step RT-PCR Kit	100 reactions	4442135
Path-ID Multiplex One-Step RT-PCR Kit	500 reactions	4442136
Path-ID Multiplex One-Step RT-PCR Kit	1,000 reactions	4442137
VetMAX-Plus One-Step RT-PCR Kit	100 reactions	4415328
VetMAX-Plus Multiplex One-Step RT-PCR Kit	100 reactions	4415330
VetMAX-Plus qPCR Master Mix	100 reactions	4415327

Find out more at thermofisher.com/animalhealth