



ThermoFisher
SCIENTIFIC



Master mixes to help meet your specific needs, sample type, and testing method

Enjoy the confidence that comes with using Applied Biosystems™ master mixes that veterinary research labs around the world have come to trust for reliability and robust performance. Whether you're working with RNA or DNA, performing singleplex or multiplex reactions, or testing simple or difficult samples, we have the optimal master mix that can work for you and help deliver robust and consistent results you can trust.

Applied Biosystems™ kits and master mixes

Fast-cycling multiplex one-step RT-PCR master mix	• VetMAX™ Fast Multiplex Master Mix
One-step RT-PCR master mix	• AgPath-ID™ One-Step RT-PCR Kit
Standard-cycling multiplex one-step RT-PCR master mix	• Path-ID™ Multiplex One-Step RT-PCR Kit
qPCR master mix	• Path-ID™ qPCR Master Mix (just for DNA)
Master mixes with internal positive control	• VetMAX™-Plus One-Step RT-PCR Kit • VetMAX™-Plus Multiplex One-Step RT-PCR Kit • VetMAX™-Plus qPCR Master Mix

applied biosystems

Fast multiplex master mix (with ROX)

VetMAX Fast Multiplex Master Mix—highly analytically sensitive master mix compatible with fast cycling conditions and optimized for inhibitory animal samples.

- Compatible with fast cycling (<50 minute thermal cycler run time)
- Compatible with a variety of assays and complexities (RNA, DNA, singleplex, multiplex)
- Ability to tolerate inhibitors from a variety of sample types such as feces, environmental samples, blood, milk, and oral fluid
- Single-tube format for ease of use

Performance

VetMAX Fast Multiplex Master Mix (with ROX) is a fast-cycling, multiplex-compatible master mix that has enhanced multiplexing capability, analytical sensitivity, and inhibitor tolerance. Figure 1 shows a comparison between a standard-cycling master mix and the VetMAX Fast Multiplex Master Mix for bovine viral diarrhea virus (BVDV) RNA. This master mix helps decrease time-to-results while providing higher-order multiplexing capabilities and enhanced inhibitor tolerance, so you can get information for up to four targets from a single reaction regardless of sample type. The single-tube format saves hands-on time—just add your assay and sample and you're ready to go.

Formulation

Fast multiplex master mix containing:

- A concentrated reverse transcriptase enzyme capable of producing high cDNA yields
- Concentrated ultrapure hot-start DNA polymerase providing exceptional analytical specificity and sensitivity
- Fast cycling–optimized 2X RT-PCR buffer for inhibitor-tolerant, multiplex-compatible reverse transcription and PCR
- Includes ROX dye as an internal reference for normalization and precise data analysis

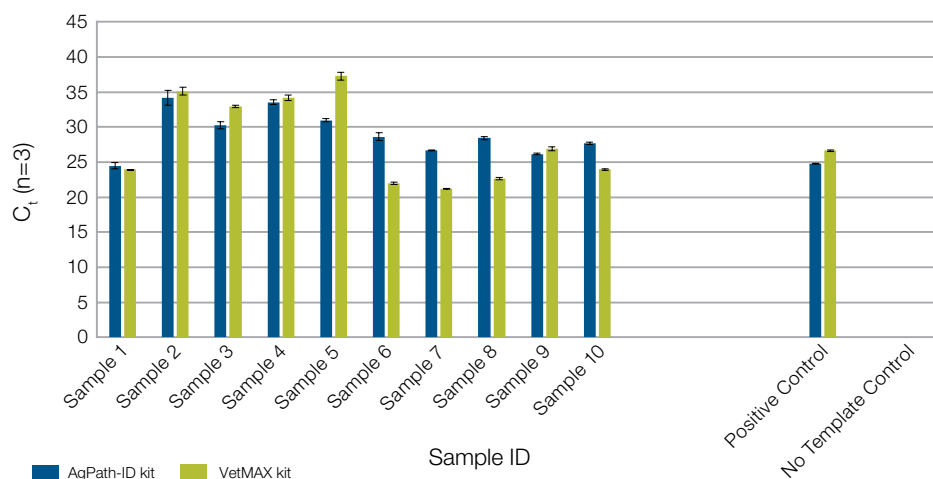


Figure 1. AgPath-ID One-Step RT-PCR Kit run on standard mode and VetMAX Fast Multiplex Master Mix (BVDV RNA) run on fast mode. These data show that the VetMAX kit has similar sensitivity compared with the AgPath-ID kit, with a significantly shorter runtime.

One-step RT-PCR master mix

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

Formulation

The AgPath-ID One-Step RT-PCR Kit is designed for analytically 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 reverse transcriptase enzyme RT that produces high cDNA yields
 - Ultrapure hot-start DNA polymerase providing exceptional analytical specificity and sensitivity
- Optimized 2X RT-PCR buffer for efficient, robust reverse transcription and PCR
 - Includes ROX dye as an internal reference for normalization and precise data analysis

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.

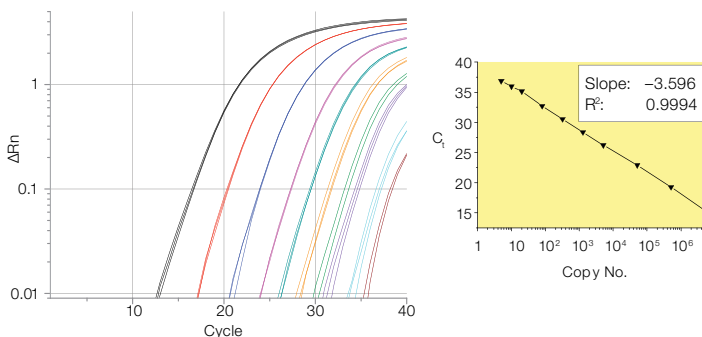


Figure 2. qRT-PCR targeting serially diluted virus A control RNA transcript (5 to 5×10^6 copies) demonstrates highly efficient and consistent performance of the AgPath-ID One-Step RT-PCR Kit.

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 another supplier's RT-PCR kit. The AgPath-ID One-Step RT-PCR Kit provided earlier C_t values and better analytical sensitivity than the other supplier's kit across the dilution range.

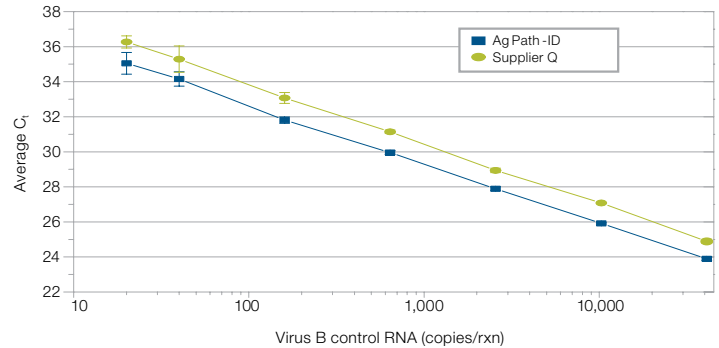


Figure 3. The AgPath-ID One-Step RT-PCR Kit is more sensitive than another leading supplier's kit. Serially diluted virus B control RNA (20 to 40,000 copies) was amplified using the AgPath-ID One-Step RT-PCR Kit and another supplier's kit.

Multiplex one-step RT-PCR master mix

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

- Simultaneous multiplex amplification of up to four different targets helps save time and money
- Optimized to amplify low-copy number (20 copies) targets to deliver results even with challenging samples
- Capable of amplification of over seven logarithmic units of input to provide robust performance when you need it

Formulation

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

- Multiplex enzyme mix containing:
 - A concentrated reverse transcriptase enzyme capable of producing high cDNA yields
 - Ultrapure hot-start DNA polymerase providing exceptional analytical 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 another supplier's product.

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 multiplexing.

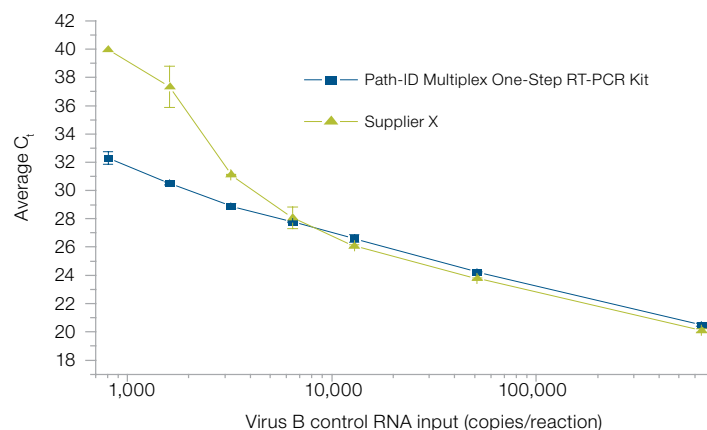


Figure 4. The Path-ID Multiplex One-Step RT-PCR Kit amplifies lower amounts of target with better analytical sensitivity (lower C_t values) than another supplier's kit. A quadruplex RT-PCR experiment was performed using the Path-ID Multiplex One-Step RT-PCR Kit and another supplier's kit. Only data for the virus B target are shown.

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

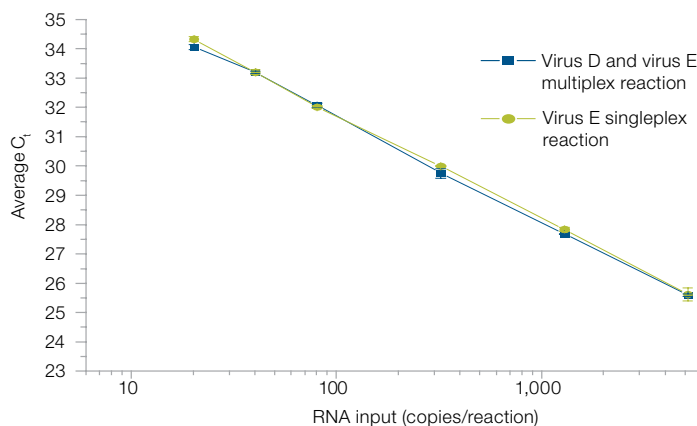


Figure 5. The Path-ID Multiplex One-Step RT-PCR Kit shows no difference in analytical sensitivity between singleplex and multiplex reactions. Virus E RNA was reverse-transcribed and PCR-amplified in a singleplex reaction, and virus D RNA and virus E RNA were reverse-transcribed and coamplified in a duplex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

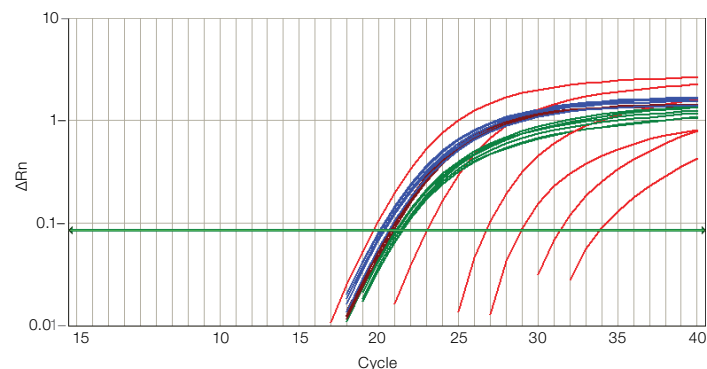


Figure 6. The Path-ID Multiplex One-Step RT-PCR Kit consistently amplifies multiple pathogen targets in a single reaction.

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. A sample set with fixed amounts of three of the targets and a serial dilution series of the virus B control RNA (red curve) were included.

qPCR master mix

Path-ID qPCR Master Mix—highly sensitive master mix used to detect animal sample–derived DNA, optimized to perform in the presence of challenging qPCR inhibitors.

- Capable of amplifying over 7 logarithmic units 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:

- Ultrapure hot-start DNA polymerase to enable room-temperature reaction setup and minimize nonspecific PCR products
- Optimized buffer and dNTPs for enhanced analytical sensitivity and functionality in the presence of PCR inhibitors
- ROX dye as an internal reference for normalization and precise data analysis

Convenience and performance

The Path-ID qPCR Master Mix provides dependable target amplification over a linear dynamic range of 6 orders of magnitude, down to 25 copies of target (Figure 7). Path-ID qPCR Master Mix enables amplification of even the most dilute samples.

Path-ID qPCR Master Mix provides reliable amplification of numerous animal sample–derived 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 μ M) and humic acid (15 ng/ μ L) compared to another supplier's master mix.

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.

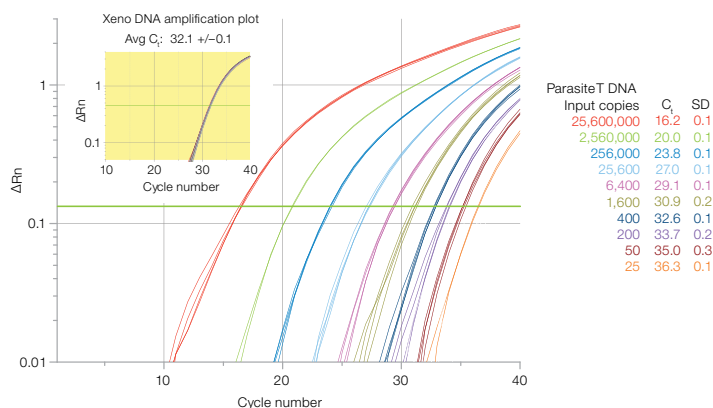


Figure 7. An amplification plot for parasite T DNA in four replicate reactions using Path-ID qPCR Master Mix demonstrates that even the most dilute samples are easily amplified. All reactions showed consistent amplification of Xeno DNA Control, an internal positive control (inset).

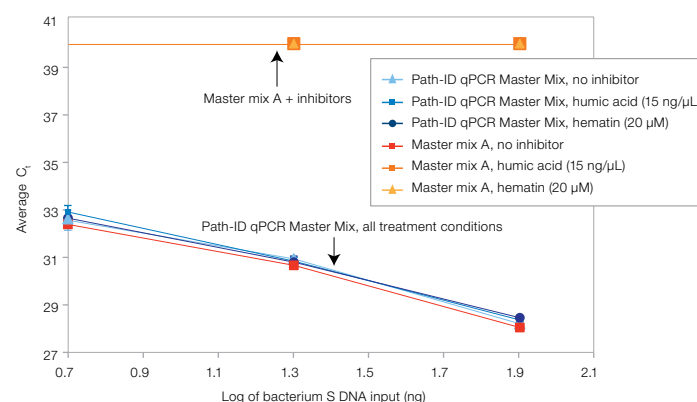


Figure 8. Path-ID qPCR Master Mix shows better tolerance to inhibitors than the competitor's master mix. C_t values are shown for amplification of a dilution series of bacterium S target DNA in the presence of PCR inhibitors, hematin (20 μ M) and humic acid (15 ng/ μ L). The limit of detection for C_t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

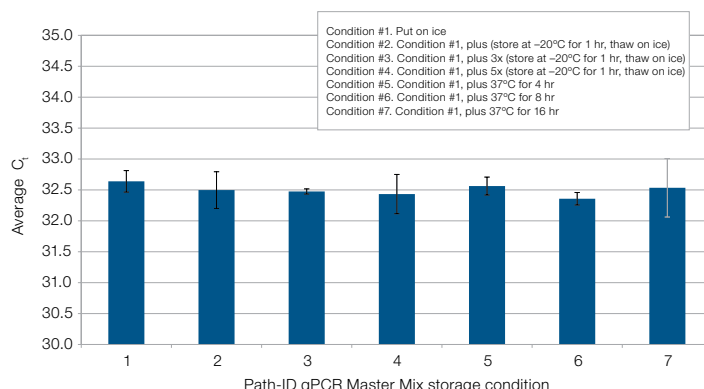


Figure 9. C_t values are given for amplification of bacterium M DNA using Path-ID qPCR Master Mix with various handling conditions. PCR was performed on bacterium M DNA using Path-ID qPCR Master Mix that had been subjected to various freeze/thaw cycles and stored at 37°C for different lengths of time.

Master mixes with internal positive control

Applied Biosystems™ VetMAX™-Plus master mixes provide the highly analytically sensitive and robust performance you need with the added confidence and convenience of a 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 sample-derived RNA or DNA
- A suite of master mix options (RT-PCR, multiplex, qPCR) is available to fit your unique application

Formulations

Components of each VetMAX-Plus kit are provided below.

VetMAX-Plus One-Step RT-PCR Kit

- 25X RT-PCR enzyme mix containing:
 - ArrayScript Reverse Transcriptase, a mutant reverse transcriptase enzyme that produces high cDNA yields
 - Ultrapure hot-start DNA polymerase providing exceptional analytical 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

VetMAX-Plus Multiplex One-Step RT-PCR Kit

- 10X multiplex enzyme mix containing:
 - A concentrated reverse transcriptase enzyme capable of producing high cDNA yields
 - Ultrapure hot-start DNA polymerase providing exceptional analytical 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

VetMAX-Plus qPCR Master Mix

- 2X qPCR master mix containing:
 - Ultrapure hot-start DNA polymerase that 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. The data show that Xeno IPC follows the target's trend of increasing C_i values due to inhibition and therefore can be used as an indicator of inhibition in the reaction. Since the expected range of Xeno IPC C_i 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.

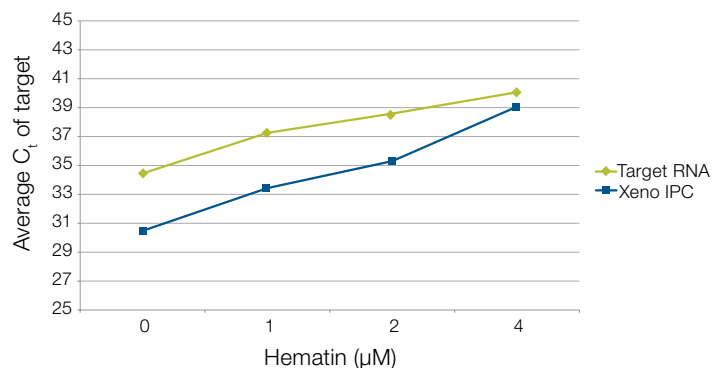


Figure 10. Graph depicting the effect of increasing inhibition on RNA target and subsequent effect on Xeno IPC. 100 copies per reaction of RNA target and 1,000 copies per reaction of Xeno IPC were exposed to increasing levels of hematin (0–4 μM).

For greater quality and consistency of sample-derived RNA and DNA detection, use VetMAX-Plus master mixes with Applied Biosystems™ VetMAX™ reagents and controls.

Ordering information

Product	Quantity	Cat. No.
Path-ID qPCR Master Mix	100 reactions	4388643
	500 reactions	4388644
AgPath-ID One-Step RT-PCR Kit	100 reactions	AM1005
	500 reactions	4387424
	1,000 reactions	4387391
Path-ID Multiplex One-Step RT-PCR Kit	100 reactions	4442135
	500 reactions	4442136
	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
VetMAX Fast Multiplex Master Mix	100 reactions	A57081
	500 reactions	A57305
	1,000 reactions	A57306

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