

TaqMan Fast Virus 1-Step Master Mix

Quantification of RNA and DNA viruses even in the presence of inhibitors



Key features

- One-tube, one-step 4X master mix to amplify both RNA and DNA with high sensitivity
- Formulated to handle common RT-PCR inhibitors found in blood, stool, and other difficult samples
- Increased qRT-PCR speed on fast and standard instruments
- Single run profile compatible with RNA and DNA and on any supported instrument, which allows for easy mix-and-match of targets on a plate
- Works in singleplex and multiplex, and with exogenous or endogenous internal controls

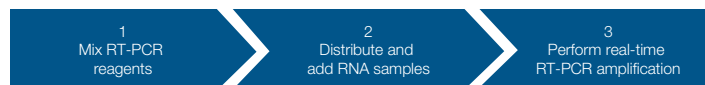
Introduction

Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix is designed for reliable, high-sensitivity real-time RT-PCR even in the presence of common reaction inhibitors. The features of the kit have been selected to optimize virus detection in commonly used sample types. A single-tube format allows for uniform handling and processing of both RNA and DNA viruses.

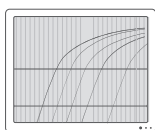
Formulation

With the TaqMan Fast Virus 1-Step Master Mix you can perform reverse transcription (RT) and PCR all in one reaction well. It includes:

- Applied Biosystems™ AmpliTaq™ Fast DNA Polymerase UP (Ultra Pure), for rapid hot-start PCR
- A rapid thermostable moloney murine leukemia virus (MMLV)-RT for high sensitivity on viral nucleic acid targets
- Additives to greatly improve success with samples that contain RT-PCR inhibitors, such as blood, anticoagulants, dirt, and stool
- A buffer solution that does not freeze at the -20°C storage temperature



TaqMan Fast Virus 1-Step Master Mix and PCR primers and TaqMan probe for your targets



Sensitivity

Even very low amounts of viral nucleic acid can be detected with the TaqMan Fast Virus 1-Step Master Mix. Using this more concentrated master mix, you can set up smaller reactions and perform fast cycling protocols with sensitivity comparable to that obtained with standard-cycling qPCR. Alternatively, larger sample input amounts can be added to standard reaction volumes for more accurate quantification of low-titer samples (Figure 1).

Using the same amount of input template, the TaqMan Fast Virus 1-Step Master Mix shows improved sensitivity when compared to other vendors' reagents, especially other fast-cycling products (Figure 2).

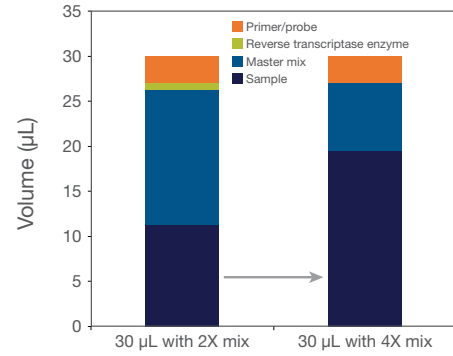
Consistent results in the presence of inhibitors

Research samples commonly assayed for viruses include blood, dirt, and tissues. Buffer components and proprietary additives in the TaqMan Fast Virus 1-Step Master Mix have been optimized to handle RT-PCR inhibitors (Figure 3) to help ensure consistent performance even with these difficult samples, so you can be more confident in your results.

Flexibility in targets

It is common for labs to test for both RNA and DNA viruses in a variety of samples. To simplify your experiments, a single TaqMan Fast Virus 1-Step Master Mix protocol has been developed to assay both types of nucleic acids, so you can perform RNA and DNA virus queries next to each other on the same plate using the same handling steps (Figure 4).

Additionally, because virus research often includes multiplexed primers and probes and internal reaction controls, we have optimized the master mix to work with multiple targets (Figure 5).



	30 µL with 2X mix	30 µL with 4X mix
Sample	11.25 µL	19.5 µL
Master mix	15 µL	7.5 µL
Reverse transcriptase	0.75 µL	Pre-mixed
Primer/probe	3 µL	3 µL

Figure 1. Comparison of sample volumes in reactions with a 2X master mix vs. a 4X master mix. Using the TaqMan Fast Virus 1-Step Master Mix at 4X concentration allows you to add almost twice as much sample to a reaction, compared to a 2X master mix. This helps to enhance sensitivity with samples containing low concentrations of viral nucleic acids.

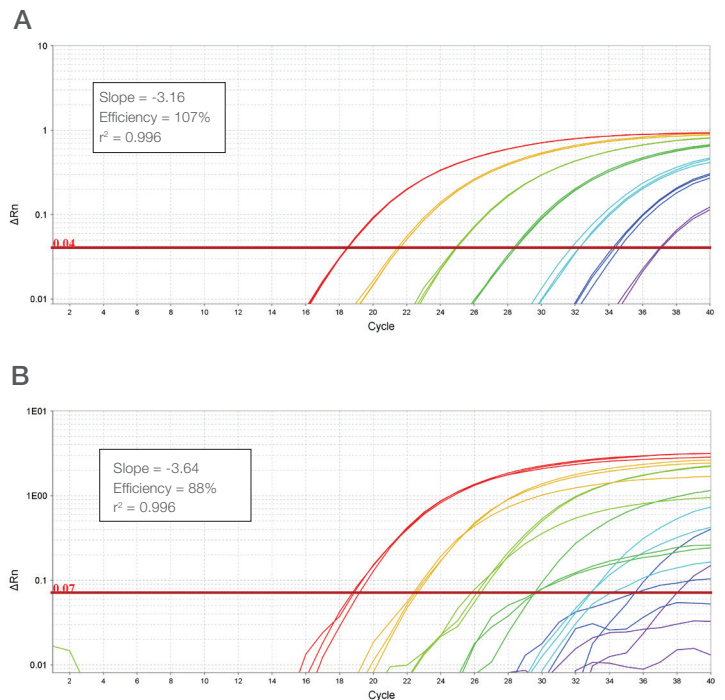


Figure 2. Comparison of sensitivity of RNA virus detection in a dilution series. The TaqMan Fast Virus 1-Step Master Mix (A) shows improved sensitivity and RT-PCR efficiency with a dilution series of an RNA viral target, compared to another vendor's fast-cycling master mix (B). Shown is a 10-fold dilution series of RNA virus, starting with 100 ng of template, run on an Applied Biosystems™ ViiA™ 7 Real-Time PCR System.

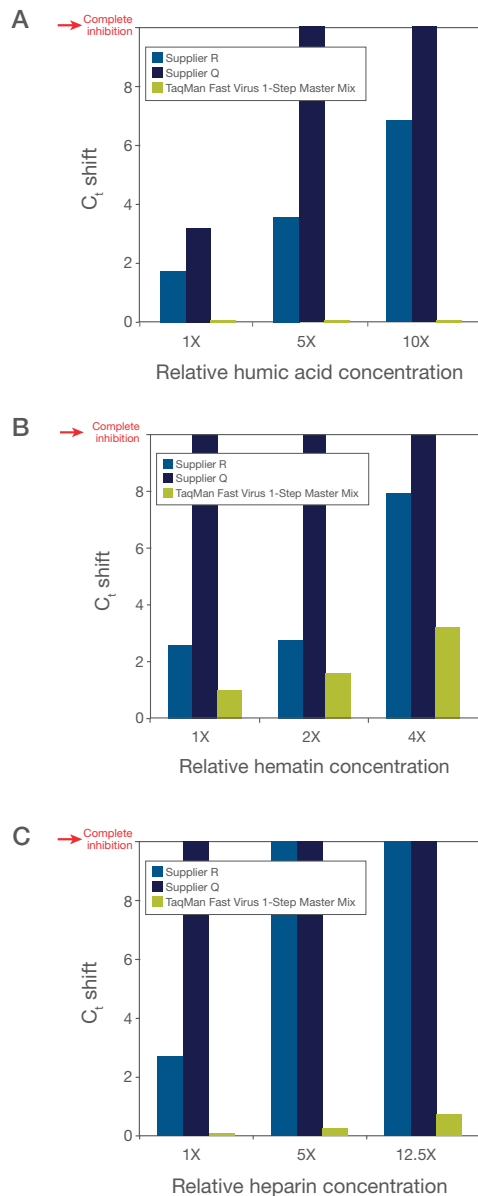


Figure 3. Comparison of inhibitor tolerance of TaqMan Fast Virus 1-Step Master Mix and one-step kits made by other vendors. Three inhibitors of RT-PCR (A, humic acid; B, hematin; C, heparin) were added to real-time RT-PCR reactions at three different concentrations with a viral target to assess the magnitude of C_t shift caused by these inhibitors. Graphs show the change in C_t from a baseline value with no inhibitor present. The TaqMan Fast Virus 1-Step Master Mix is clearly more robust to humic acid, heparin, and hematin, and real-time RT-PCR results are often achievable with minimal loss of sensitivity, even at concentrations that will completely inhibit other products.

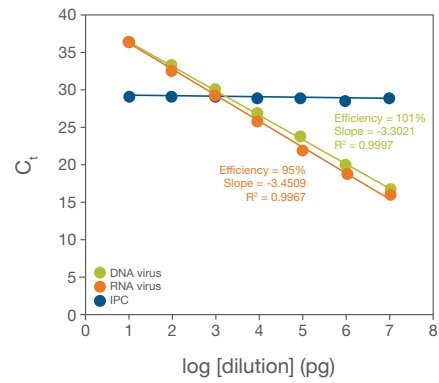


Figure 4. TaqMan Fast Virus 1-Step Master Mix in triplex. Two viral targets and an exogenous internal positive control (IPC) were run together in the same well. One target was an RNA virus, the other was a DNA virus, and the IPC was an RNA target. With a single mix optimized to both RNA and DNA viral targets in singleplex or multiplex, it is possible to maximize the efficiency of your real-time PCR instrument.

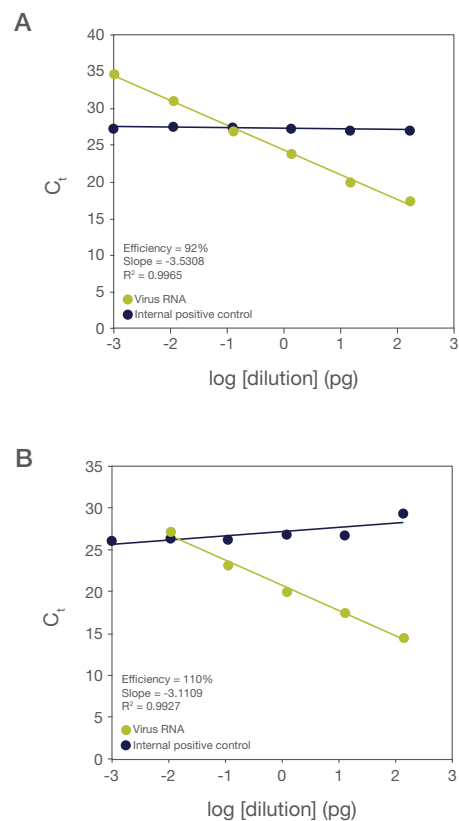


Figure 5. TaqMan Fast Virus 1-Step Master Mix sensitivity in duplex. Real-time PCR reactions were performed with a viral target and an exogenous internal positive control (IPC). The virus concentration was titrated over a constant concentration of IPC. TaqMan Fast Virus 1-Step Master Mix (A) shows better sensitivity in the duplex reaction than another vendor's one-step kit (B)—the final dilution was detectable in the linear range, rather than being undetectable. Additionally, the reaction shows superior stability of the IPC C_t over the full dynamic range of the viral nucleic acid titration.

Fast

The TaqMan Fast Virus 1-Step Master Mix speeds your time-to-results and maximizes the use of your real-time PCR instruments. The 4X formulation allows for more target nucleic acid sample to be added to the smaller reaction volumes (required for fast protocols). This enables you to maintain sensitivity with low-titer research samples while improving speed and throughput (Figure 6).

Conclusion

TaqMan Fast Virus 1-Step Master Mix is a reliable, efficient, and accurate reagent for real-time RT-PCR of virus samples. Its robust performance in the presence of common RT-PCR inhibitors and its convenient and flexible reaction setup allows you to have more confidence in your results.

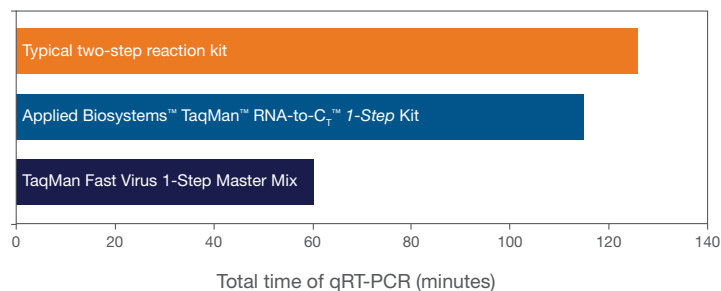


Figure 6. Experiment times for three RT-PCR kits from Thermo Fisher Scientific. For kits that allow a fast cycling protocol, such as the TaqMan Fast Virus 1-Step Master Mix, it is possible to perform twice as many runs with the same instrument as can be completed with a standard cycling reagent in the same time. Additionally, compared to other one-step kits, the single-tube format of the TaqMan Fast Virus 1-Step Master Mix saves hands-on time.

Ordering information

	Size	Quantity	No. of 20 μ L reactions	Cat. No.
TaqMan Fast Virus 1-Step Master Mix	1-pack	1 x 1 mL	200	4444432
	5-pack	5 x 1 mL	1,000	4444434
	Bulk pack	1 x 10 mL	2,000	4444436

Custom formulation with uracil-DNA glycosylase (UDG) premixed also available. Please inquire with your sales representative.

Find out more at thermofisher.com/qpcrmm

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