Real-time PCR

TaqMan Fast Virus 1-Step master mixes

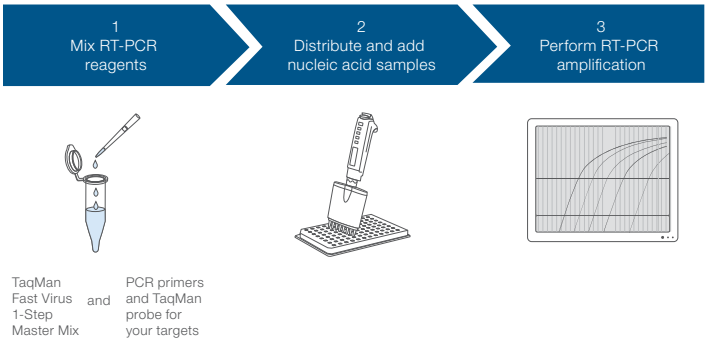
Quantification of RNA and DNA viruses even in the presence of inhibitors

Key features

- One-step real-time PCR master mixes to detect both viral RNA and DNA with high sensitivity
- Single-tube master mix provided in a 4X concentration to enable more sample to be run per reaction
- Formulations available with and without ROX™ reference dye to enable multiplexing flexibility
- Formulated to handle common reverse transcription PCR (RT-PCR) inhibitors found in blood, stool, and other difficult samples
- Increased RT-PCR speed on fast and standard instruments
- Compatible with running RNA and DNA simultaneously on any supported instrument, which allows for easy mix-and-match of targets on a plate
- Can be run in single-plex and multiplex reactions and with exogenous or endogenous internal controls

Introduction

Applied Biosystems™ TaqMan™ Fast Virus 1-Step master mixes are designed for reliable, high-sensitivity real-time RT-PCR, even in the presence of common reaction inhibitors. The formulation of this family of master mixes has been optimized to detect viruses in commonly used sample types. A single-tube format allows for uniform handling and processing of both RNA and DNA viruses.



Formulation

With TaqMan Fast Virus 1-Step master mixes, you can perform reverse transcription and quantitative PCR all in one reaction well. The mixes include:

- Applied Biosystems™ AmpliTaq™ Fast DNA Polymerase UP (Ultra Pure), for rapid hot-start PCR
- A fast, thermostable Moloney murine leukemia virus (MMLV) reverse transcriptase with high sensitivity for viral nucleic acid targets
- Additives to improve success with samples that contain RT-PCR inhibitors, such as blood, anticoagulants, dirt, and stool
- A buffer solution that does not freeze when stored at –20°C
- ROX passive reference dye (if applicable)

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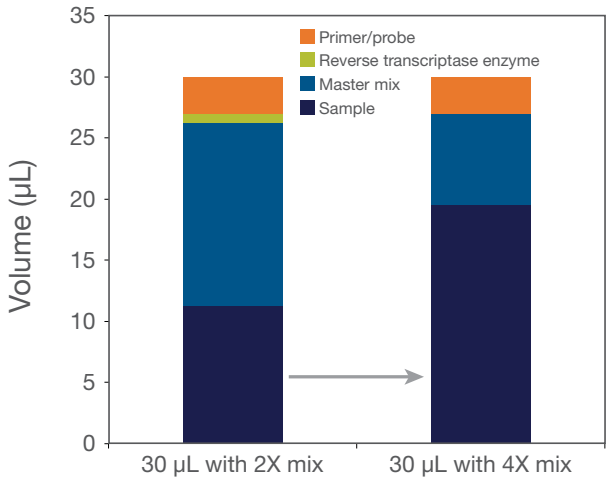
Sensitivity

Even very low amounts of viral nucleic acid can be detected with 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, TaqMan Fast Virus 1-Step Master Mix shows improved sensitivity when compared to other vendors' reagents, especially other fast-cycling products (Figure 2).

TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX) demonstrates similar sensitivity, detecting down to 5 copies of target template per reaction (Figure 3).



	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	Premixed
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 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 for samples containing low concentrations of viral nucleic acids.

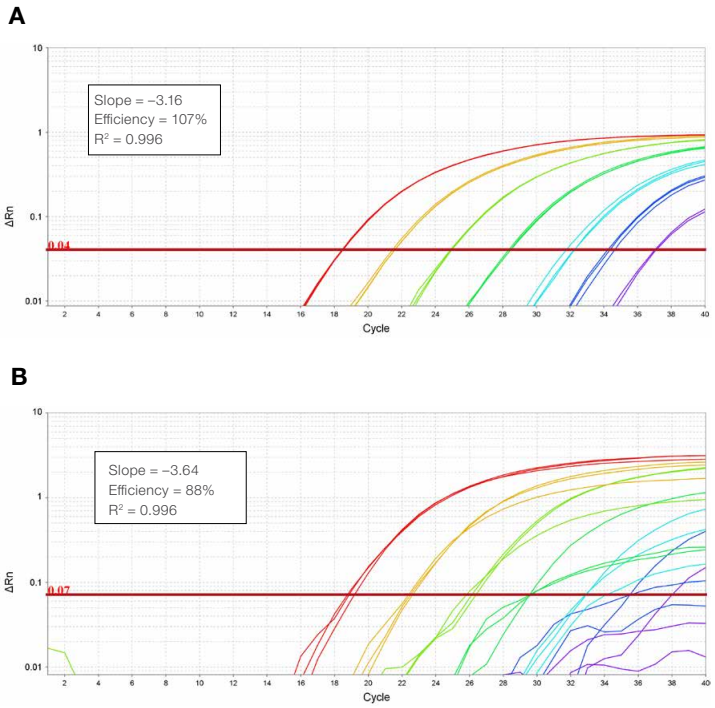


Figure 2. Comparison of master mix sensitivity for RNA virus detection in a dilution series. (A) TaqMan Fast Virus 1-Step Master Mix shows improved sensitivity and RT-PCR efficiency with a dilution series of a viral RNA target, compared to **(B)** another vendor's fast-cycling master mix. Shown is a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

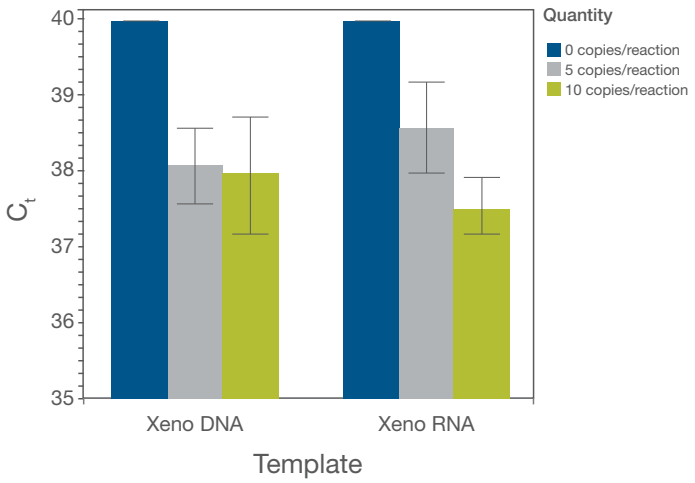


Figure 3. Low-copy detection with TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX). Xeno DNA (left) and xeno RNA (right) were detected using TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX) and an assay labeled with FAM™ dye. Samples contained 0 copies/reaction (no-template control), 5 copies/reaction, or 10 copies/reaction of either xeno DNA or RNA. Error bars are 95% confidence interval from the mean of 16 replicates. Reactions with 10 or 5 copies/reaction are significantly different from the no-template control.

Multiplexing capability

Because virus research often includes multiplexed primers and probes and internal reaction controls, we have optimized the master mixes to work with multiple targets. TaqMan Fast Virus 1-Step master mixes demonstrate exceptional sensitivity in commonly used duplex reactions (Figure 4). For higher-order multiplexing, TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX) has increased flexibility to accommodate four targets in a single reaction well (Figure 5). By removing the ROX passive reference dye, this formulation of the master mix enables customers to use other dyes, such as JUN™ dye, in the channel.

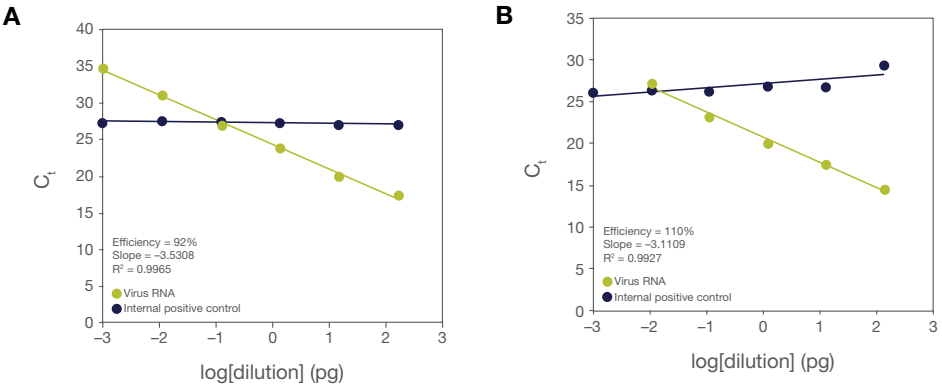


Figure 4. TaqMan Fast Virus 1-Step Master Mix sensitivity in a duplex reaction. Real-time PCR reactions were performed with a viral target and an exogenous internal positive control (IPC). The virus concentration was titrated over a constant concentration of IPC. **(A)** TaqMan Fast Virus 1-Step Master Mix shows better sensitivity in the duplex reaction than **(B)** another vendor's one-step kit—the final dilution was detectable in the linear range, rather than being undetectable. Additionally, the reaction shows superior stability of the IPC C_t values over the full dynamic range of the viral nucleic acid titration.

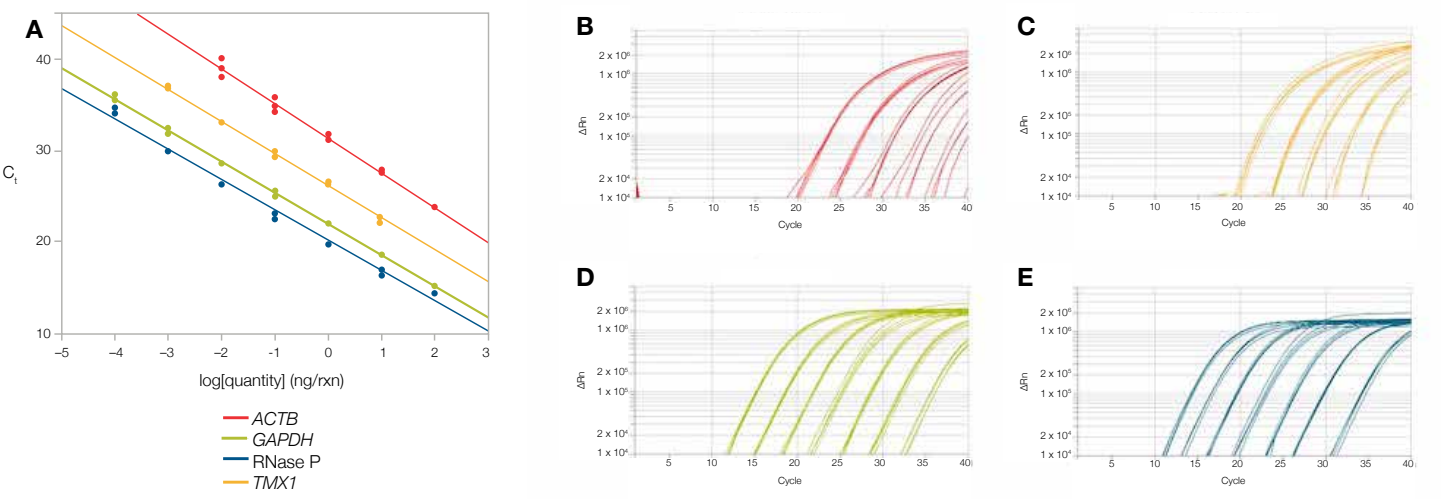


Figure 5. TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX) was developed for higher-order multiplex reactions. (A) Linear fit of *ACTB*, *GAPDH*, RNase P gene, and *TMX1* targets for a 4-plex reaction with universal human reference RNA, TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX), and TaqMan Gene Expression Assays. R^2 = 1.0 for all targets. Amplification is observed over 6 logarithmic units for *GAPDH* and the RNase P gene. Amplification is observed over 4 logarithmic units for challenging targets (*ACTB* and *TMX1*). Amplification curves for **(B)** *ACTB*, **(C)** *TMX1*, **(D)** *GAPDH*, and **(E)** the RNase P gene.

Flexibility with RNA and DNA targets

It is common for laboratories 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 in the same plate or the same well using the same handling steps (Figure 6).

Consistent results in the presence of inhibitors

Research samples commonly assayed for viruses include blood, dirt, and tissues. Buffer components and proprietary additives in TaqMan Fast Virus 1-Step Master Mix have been optimized to handle RT-PCR inhibitors (Figure 7) to help ensure consistent performance even with these difficult samples, so you can be more confident in your results.

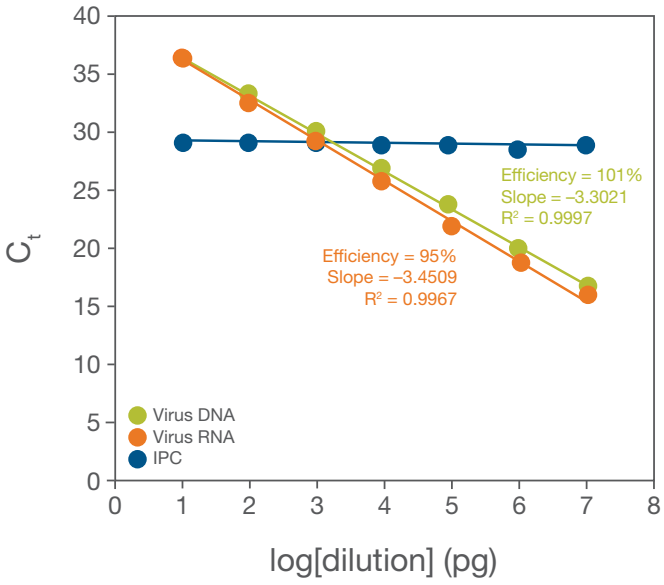


Figure 6. TaqMan Fast Virus 1-Step Master Mix in a triplex reaction with RNA and DNA targets. Two viral targets and an exogenous IPC were run together in the same well. The three targets were virus RNA, virus DNA, and an RNA target as an IPC. With a single mix optimized for both RNA and DNA viral targets in single-plex or multiplex reactions, it is possible to maximize the efficiency of your real-time PCR instrument.

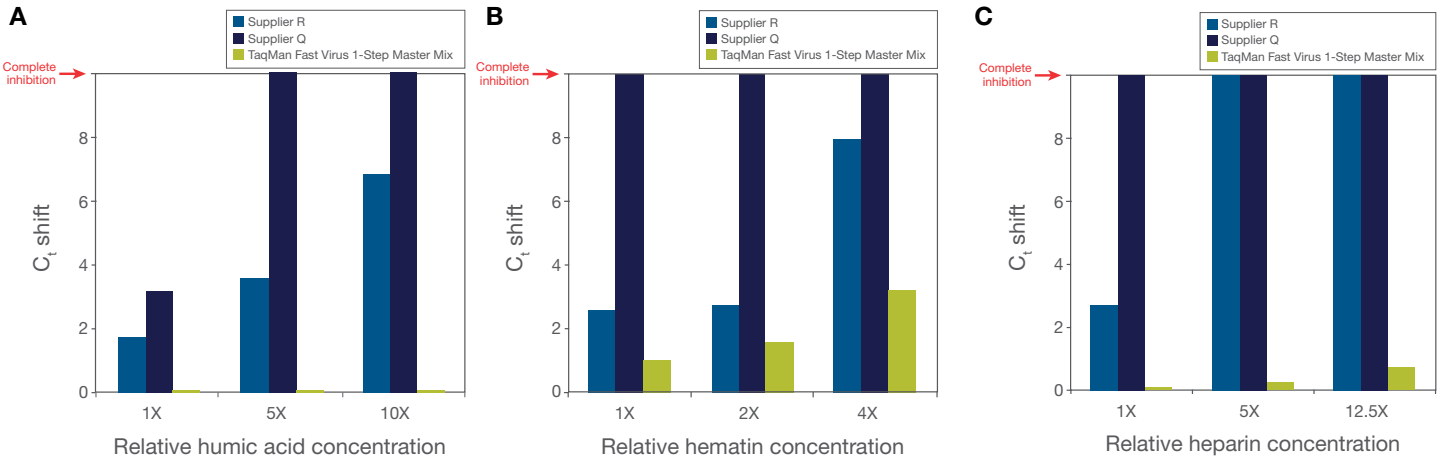


Figure 7. Comparison of inhibitor tolerance of TaqMan Fast Virus 1-Step Master Mix and one-step kits made by other vendors. Three inhibitors of RT-PCR—**(A)** humic acid, **(B)** hematin, and **(C)** heparin—were added to real-time RT-PCR reactions at three different concentrations with a viral target to assess the magnitude of C_t shift caused by these inhibitors. Graphs show the change in C_t values from a baseline value resulting from a reaction with no inhibitor. The TaqMan Fast Virus 1-Step Master Mix is more resistant to humic acid, heparin, and hematin, and real-time RT-PCR results are often achievable with minimal loss of sensitivity, even at concentrations that completely inhibit other products. Similar performance results in the presence of inhibitors were obtained for TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX). See the **product page** for experiment details.

Fast

TaqMan Fast Virus 1-Step master mixes speed your time-to-results and maximize the use of your real-time PCR instruments. The 4X formulation allows for more target nucleic acid sample to be added to smaller reaction volumes (required for fast protocols). This enables you to maintain sensitivity with low-titer research samples while improving speed and throughput (Figure 8).

Conclusion

TaqMan Fast Virus 1-Step master mixes are reliable, efficient, and accurate reagents for real-time RT-PCR using virus samples. This family of master mixes features robust performance in the presence of common RT-PCR inhibitors and a convenient and flexible reaction setup to help you have more confidence in your results.

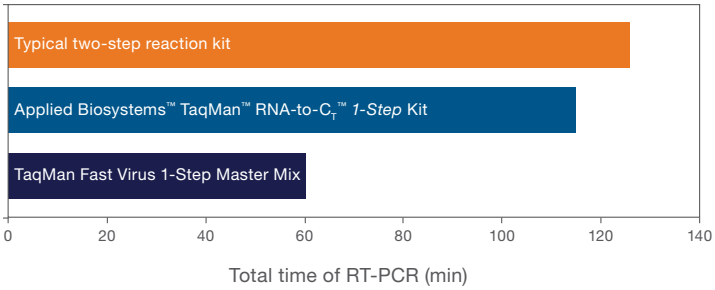


Figure 8. Experiment times for three RT-PCR kits from Thermo Fisher Scientific. For kits that allow a fast cycling protocol, such as the TaqMan Fast Virus 1-Step Master Mix, it is possible to perform twice as many runs with the same instrument as can be completed with a standard-cycling reagent in the same time. Additionally, compared to other one-step kits, the single-tube format of TaqMan Fast Virus 1-Step Master Mix saves hands-on time.

Ordering information

Product*	Quantity	No. of 20 µL reactions	Cat. No.
TaqMan Fast Virus 1-Step Master Mix	1 x 1 mL	200	4444432
	5 x 1 mL	1,000	4444434
	1 x 10 mL	2,000	4444436
TaqMan Fast Virus 1-Step Multiplex Master Mix (No ROX)	1 x 1 mL	200	5555532
	5 x 1 mL	1,000	5555534
	1 x 10 mL	2,000	5555536

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

Learn more at **thermofisher.com/taqman-fast-virus-1-step-master-mixes**

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