Compare your current 1-step probe mix with Applied Biosystems™ TaqMan™ Fast Virus 1-Step Multiplex Master Mix



Assess performance for your 1-step RT-qPCR workflow using a simple side-by-side serial dilution experiment

Efficiency
Calculated from the standard curve slope

Linearity
R^2 value across the dilution series

Precision
Replicate C_t consistency at each dilution

Analytical ensitivity
Lowest input with reproducible detection

General guidelines

- Consider testing multiple assays across a range of expression levels
- Use the manufacturer's recommended assay, template, and cycling conditions
- Keep plate format, reaction volume, and analysis settings consistent where possible
- Include a no-template control for each assay
- For multiplex evaluation, confirm singleplex performance first
- Run reactions in triplicate to measure precision

Step 1: prepare a dilution series

Prepare a 10-fold dilution series from a single RNA sample source. For comparison, the same dilution point can be used for all assays. This example provides enough volume to run one dilution point in triplicate for each real-time PCR (qPCR) assay mix, for up to four assay mixes (see diagram on page 2).

Dilution	Template	Nuclease-free water
Undiluted	100 µL DNA template	-
10^{-1}	10 µL of undiluted	90 µL
10^{-2}	10 µL of 10^{-1}	90 µL
10^{-3}	10 µL of 10^{-2}	90 µL
10^{-4}	10 µL of 10^{-3}	90 µL
10^{-5}	10 µL of 10^{-4}	90 µL
10^{-6}	10 µL of 10^{-5}	90 µL

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Step 2: set up the reactions

The reaction setup below uses a 20X Applied Biosystems™ TaqMan™ Assay (primer and probe) format in a 96-well plate with a 20 µL reaction volume. For other primer/probe assay formats, follow the manufacturer's recommended setup.

1. For the first assay, use the table below to prepare enough TaqMan Fast Virus 1-step reaction mix for 26 reactions (20 µL each). Mix thoroughly.

Component	Volume for 26 reactions (24 reactions + overage)
TaqMan Fast Virus 1-Step Multiplex Master Mix (4X)	130 µL
Assay 1 (20X)	26 µL
Nuclease-free water	234 µL
Total volume	**390 µL**

Note: for 10 µL reactions in a 384-well plate, reduce all volumes by half.

2. Pipette 15 µL of the reaction mix containing TaqMan Fast Virus 1-Step Multiplex Master Mix into the appropriate wells of a 96-well optical plate, according to the plate map below.

3. Add 5 µL of each template dilution from step 1 into the appropriate wells, according to the plate map below. For no-template control (NTC) reactions, use 5 µL of nuclease-free water.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	1X	1X	1X	1X	1X	1X	1X	1X	1X	1X	1X	1X	A
B	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	B
C	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	C
D	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	D
E	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	E
F	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	F
G	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	G
H	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Assay 1 **Assay 2** **Assay 3** **Assay 4**

4. Repeat steps 1–3 for any additional qPCR mixes, up to four assays per plate.

5. Seal the plate, then briefly centrifuge.

6. Start your qPCR run.

7. If comparing performance to another master mix, repeat steps 1–6 using the other master mix and a new plate.

Step 3: run qPCR

Run each plate according to the cycling conditions provided in the reagent user documentation.

Fast cycling conditions for TaqMan Fast Virus 1-Step Multiplex Master Mix

Step	Temperature	Time	Cycles
Reverse transcription	50°C	5 min	Hold
Polymerase activation	95°C	20 sec	Hold
Denaturation	95°C	3 sec	40x
Anneal/extend	60°C	30 sec	

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Will these cycling conditions work with my qPCR instrument?

The TaqMan Fast Virus 1-Step Multiplex Master Mix user guide provides these cycling conditions as a starting point for most instruments, including Bio-Rad™ CFX Opus 96 and Roche™ LightCycler™ PRO systems.

Cycling conditions can vary by assay and instrument, so refer to the appropriate user documentation as needed.

Does TaqMan Fast Virus 1-Step Multiplex Master Mix contain Applied Biosystems™ ROX™ dye?

TaqMan Fast Virus 1-Step Multiplex Master Mix does not contain ROX dye. Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix is also available with ROX dye.

Some systems, such as Bio-Rad and Roche qPCR systems, do not use ROX dye for passive reference normalization.

If you are comparing a master mix without ROX dye to one containing ROX dye, you may notice differences in C_t values or baseline signal. Evaluate efficiency, precision, linearity, and sensitivity rather than relying on C_t values alone.

Refer to your instrument and analysis software documentation for dye compatibility and normalization settings

Step 4: analyze the results

Review the standard curve in your analysis software to identify the slope of the standard curve, efficiency, R^2, and replicate C_t values. Refer to your software documentation for analysis instructions.

Efficiency

Review the efficiency value calculated by your analysis software from the slope of the standard curve. Values between 90–110% are generally acceptable.

Precision

Review the standard deviation (SD) of technical replicate C_t values at each dilution. Lower SD values indicate better replicate agreement, and SD ≤0.250 is generally acceptable.

Linearity

Review the R^2 value for the standard curve across the dilution series. Values closer to 1.0 indicate better linearity, and R^2 >0.99 is generally acceptable.

Analytical sensitivity

Review the dilution series to identify the lowest dilution with reproducible amplification across replicates.

Don't be misled by lower C_t values alone. Review the full dilution series and use the evaluation scorecard on the next page to compare results.

Evaluation scorecard

Use the scorecard below to compare performance for each assay across both qPCR mixes. When reviewing the dilution series, note that the usable dynamic range is defined by the portion of the dilution series where both amplification efficiency and linearity remain acceptable.

Tip: use the "Notes" column to capture observations from the dilution series, including the range where efficiency and linearity remain acceptable, replicate variability, or any setup or analysis issues.

Assay		Efficiency (%)	Linearity (R_2)	Precision (SD)	Analytical Sensitivity	Notes
1	Current mix					
	TaqMan Fast Virus 1-Step Multiplex Master Mix					
2	Current mix					
	TaqMan Fast Virus 1-Step Multiplex Master Mix					
3	Current mix					
	TaqMan Fast Virus 1-Step Multiplex Master Mix					
4	Current mix					
	TaqMan Fast Virus 1-Step Multiplex Master Mix					

Finished your evaluation? Choose your next step

Complete the evaluation survey

Tell us how the master mix performed and request specialist follow-up for quote support (optional).

Ready to move forward?

Visit the product page to purchase and view current promotional offers

TaqMan Fast Virus 1-Step Master Mixes

No ROX dye: 5555532, 5555534, 5555536

With ROX dye: 4444432, 4444434, 4444436

Complete your survey at
thermofisher.com/master-mix-evaluation

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