AzuraQuant 4x RT-qPCR Virus Mix

Catalog No.	Pack Size	Components and Volume	
AZ-4601	100 x 20 µL reactions	4x Mix: 1 x 0.50 mL	20x RT: 1 x 0.1 mL
AZ-4605	500 x 20 µL reactions	4x Mix: 2 x 1.25 mL	20x RT: 5 x 0.1 mL
AZ-4620	2000 x 20 µL reactions	4x Mix: 8 x 1.25 mL	20x RT: 4 x 0.5 mL
AZ-4650	5000 x 20 µL reactions	4x Mix: 5 x 5.00 mL	20x RT: 1 x 5.00 mL

Description

AzuraQuant 4x RT-qPCR Virus Mix is a high-performance 1-Step RT-qPCR master mix comprised of 4x AzuraQuant Probe Master Mix and 20x AzuraSprint Reverse Transcriptase with RNase inhibitor. The 4x master mix concentration enables higher sample input, maximizing sensitivity to low-copy RNA and DNA targets. The higher-concentration master mix also allows you to set up smaller volume reactions, enabling fast cycling protocols and excellent sensitivity. AzuraQuant 4x RT-qPCR Virus Mix is formulated for use with dual-labeled hydrolysis probes and is compatible with a wide range of fast and standard cycling conditions in singlepex and multiplex PCR. The advanced 4x formulation delivers robust performance with GC-rich and AT-rich templates and is resistant to many common PCR inhibitors.

In order to maximize compatibility with higher order multiplexing (>3 targets), AzuraQuant 4x RT-qPCR Virus Mix does not contain ROX passive reference dye.

Storage

AzuraQuant 4x RT-qPCR Virus Mix is shipped on blue ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided.

Important Guidelines

- Use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMP™ (http://dnasoftware.com/). Primers should have a melting temperature (Tm) of approximately 60°C. The Tm of the probe should be approximately 6°C - 10°C higher than that of the primers. For Taqman™ probes, choose a probe close to the 5' primer and avoid terminal guanosine residues.

- Typical amplicon length should be 80 bp - 200 bp and does not usually exceed 400 bp.

- Different real-time PCR instruments require different levels of ROX™ passive reference dye. Generally, modern instruments do not require passive reference but include the option to use it for normalization. AzuraQuant 4x RT-qPCR Virus Mix does not contain ROX.

Reaction setup

1. Prepare a qPCR master mix based on following table (briefly mix 4x AzuraQuant Probe Mix by inversion):

Component	20 µL Reaction	Final Concentration/Notes
4x AzuraQuant Probe Mix	5 µL	1x
Forward Primer (10 µM)	0.8 µL	400 nM
Reverse Primer (10 µM)	0.8 µL	400 nM
Probe (10 µM)	0.4 µL	200 nM
20x AzuraSprint RTase	1 µL	Add prior to RNA
Template RNA	10 pg to 100 ng Total RNA >0.01pg mRNA	variable
PCR-grade water	Up to 20 µL final volume	

2. Program the qPCR instrument using following conditions, acquiring data on the appropriate channel:

Cycles	Temperature & Time	Notes
1	50°C, 10 minutes	cDNA synthesis
1	95°C, 1 minute	Enzyme activation
30 - 40	95°C, 5 seconds 60°C to 65°C, 20 seconds	Denaturation Anneal/Extension (do not exceed 30 seconds and do not use temps below 60°C

Quality Control

AzuraQuant 4x RT-qPCR Virus Mix is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. AzuraQuant 4x RT-qPCR Virus Mix is manufactured under a comprehensive quality management system, following ISO 9001:2015 standards.