Vita One-Step RT-qPCR Master Mix

Catalog #	Description
RTO1001	Vita One-Step RT-qPCR Master Mix, 1 ml (1 x 0.5 ml vial), 100 x 20 µl reactions
RTO1002	Vita One-Step RT-qPCR Master Mix, 5 ml (10 x 0.5 ml vials), 1000 x 20 µl reactions

For research purposes only

Product Contents

Catalog #	RTO1001	RTO1002
Preps	100	1000
4xVita One-Step RT-qPCR Master Mix	0.5 ml x 1	0.5 ml x 10
10x Reaction Buffer	0.3 ml x 1	1.5 ml x 2

Introduction

Vita One-Step RT-qPCR Master Mix is a ready-to-use 4x reaction mix optimized for RT-qPCR multiplex TaqMan probe-based detection. It contains the high efficiency MMLV Reverse Transcriptase and Taq DNA polymerase with modified 3' to 5' exonuclease activity, dNTPs, stabilizers, RNase inhibitors and RT-qPCR reaction components.

Storage and Stability

Store at –20°C, the Vita One-Step RT-qPCR Master Mix is stable for up to 12 months if stored in a constant temperature freezer at –20°C protected from light. For convenience, the master mix can be stored at 4°C during the shipping for up to 2 weeks.

Instrument Compatibility

The Vita One-Step RT-qPCR Master Mix is compatible with all Roche LightCyclers, Applied Biosystems, Bio-Rad, Eppendorf, QIAGEN real-time PCR instruments.

Vita One-Step RT-qPCR Reaction Protocol

1. Prepare the RT-qPCR reactions according to Table 1. Prepare the reaction mix with enough volume of components, to account for volume (typically 10%) loss from liquid handling, at room temperature within 10 minutes, longer time is needed, keep the reaction on ice or at 4°C.
   Prepare the RT-qPCR reactions according to Table 1

Table 1. Reaction setup

Component	Volume per 20 µL Reaction	Volume per 10 µL Reaction	Final Concentration
4x Vita One-Step RT-qPCR Master Mix	5 µL	2.5 µL	1x
Forward & reverse primers*	Variable	Variable	100-500 nM each
Fluorogenic probe*	Variable	Variable	150-250 nM each
RNA template	Variable	Variable	1 pg-1 µg
10X Reaction Buffer	2 µL	1 µL	
Nuclease-free water	Variable µL	Variable µL	
Total reaction volume	20 µL	10 µL	

2. Use purified RNA template (if needed) in nuclease-free water, to the PCR tubes or wells containing the reaction mix (Table 1), mix the reaction thoroughly to ensure homogeneity and dispense equal aliquots into each PCR tube or into the wells of a PCR plate, seal tubes or wells with flat caps or optically transparent film, gently vortex to ensure thorough mixing of the reaction components, and spin down the PCR reaction mixed tubes or plate to remove any air bubbles.

3. Setup the thermal cycling protocol on the real-time PCR instrument according to Table 2.

Table 2. Thermal cycling protocol

Step	Temperature (°C)	Duration	Number of cycles
1. Reverse transcription	55	20 min	
2. Template denaturation Annealing / extension	95 62* (optimized)	5sec 30sec	35-40 cycles

* Annealing/extension may vary by primers' Tm

4. Place the PCR tubes or plates onto the real-time PCR instrument and start the RT-qPCR run, collect and analyze data according to the instructions in the instrument-specific software.