Direct Saliva and Swab SARS-CoV-2 Vita One-Step RT-qPCR Assay

Catalog #	Description
RTSS1001	Direct Saliva and Swab Vita One-Step RT-qPCR Assay, 100 x 20 µl reactions
RTSS1002	Direct Saliva and Swab Vita One-Step RT-qPCR Assay, 1000 x 20 µl reactions

For research purposes only

Product Contents

Catalog #	RTSS1001	RTSS1002
Preps	100	1000
Lysis Buffer	1 ml x 1	1.8 ml x 6
Primers and probe mix with FAM dye (SARS-CoV-2 N1&N2 specific genes targeted)	1.3 ml x 1	1.8 ml x 8
4xVita One-Step RT-qPCR Master Mix	0.5 ml x 1	1 ml x 5
Positive RNA Control	0.1 ml x 1	0.2 ml x 4

Introduction

Direct Saliva and Swab SARS-CoV-2 Vita One-Step RT-qPCR Assay is a ready-to-use multi-target design for emerging SARS-CoV-2 variants without viral RNA purification. The assay is 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 SARS-CoV-2 RT-qPCR specific probes.

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

Steps
1. Pipet 100 µL Saliva or Swab sample into a tube and incubate at 95°C for 20 min
2. Pipet 10 µL of 95°C treated Saliva or Swab sample (avoid any precipitation) into a new tube and 10 µL of Lysis buffer to the sample and mixing
3. Pipet 2 µL of Saliva or Swab - Lysis buffer mixed sample to a new PCR tube or well of 96 wells plate
4. Add 13 µL primers and probe (SARS-CoV-2 N1 and N2 specific genes targeted) mix with FAM dye to the sample and mixing
5. Add 5 µL 4x Vita One-Step RT-qPCR Master Mix to the sample and mixing
Total reaction volume 20 µL

2. 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	5 sec 30 sec	35-40 cycles
The RT-qPCR reading in FAM dye			

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.

5. Positive RNA Control reactions according to Table 3

Table 3. Positive RNA Control Reaction setup

Steps
1. Pipet 2 µL of Positive RNA Control into a PCR tube or well
2. Add 13 µL primers and probe mix with FAM dye to the sample and mixing
3. Add 5 µL 4x Vita One-Step RT-qPCR Master Mix to the sample and mixing (Total 20 µL)
4. Proceed to the thermal cycling protocol on the real-time PCR instrument according to Table 2.