VirPath SARS-CoV-2 Multiplex qRT-PCR

Instructions For Use (IFU) v1.0.3

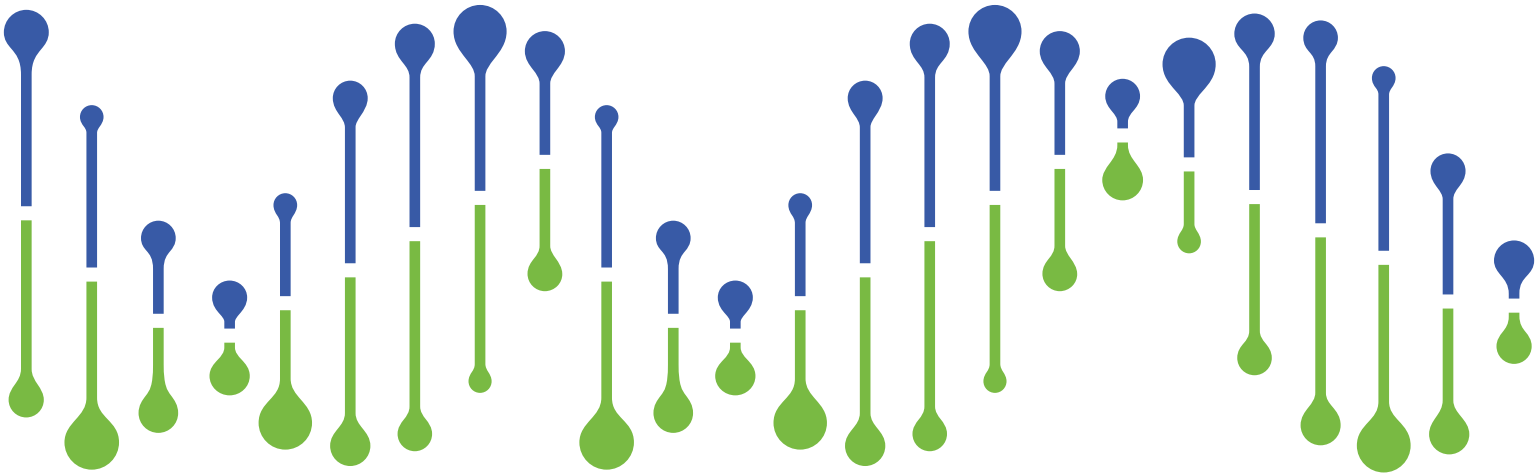
Made in the UK. Nonacus Ltd, Birmingham Research Park, Vincent Drive, Birmingham, B15 2SQ UK.

-20°C Store at -20°C

REF C3COV187
C3COV188

CE

IVD For in vitro diagnostic use



NONACUS
Advancing Non-Invasive Healthcare

Intended use

The SARS-CoV-2 multiplex qRT-PCR kit is a qualitative real-time reverse transcription polymerase chain reaction (RT-PCR) assay intended to be used for the detection of Severe Acute Respiratory Syndrome Virus coronavirus-2 (SARS-CoV-2). The kit is compatible with genomic RNA extracted from biological samples derived from lower or upper respiratory tract specimens (e.g.nasopharyngeal or oropharyngeal swabs, etc.) collected from individuals that meet the criteria for testing SARS-CoV-2 testing. The inhibitor tolerant qRT-PCR mix provided in the kit is a one-step solution designed for amplification of the SARS-CoV-2 RNA targets.

Included in the kit is a multiplex of primer / probe assays which target the nucleocapsid gene (assay N1) and the envelope gene (assay E) of SARS-CoV-2; and the RPP30 gene (assay RP) used as internal RNA extraction control. Two controls are also included in the kit to confirm functionality of the assays and the qRT-PCR reaction: the RPP30 Negative Control and the combined 2019-nCoV nucleocapsid gene and 2019-nCoV envelope gene Positive Control. The kit also contains ROX reference dye at 10x concentration for use with real-time quantitative PCR platforms that require it.

Performance has been verified using the BioRad CFX96, controlled by the CFX software. The assay should be compatible with other equivalent real-time PCR cyclers but validation should be performed prior to use.

Testing is intended for use by trained laboratory technicians who are proficient in performing molecular based tests.

Kit contents

Reagent	Volume (1,000 rxns)	Volume (10,000 rxns)	Storage
VirPath qRT-PCR Master Mix	4x 1.5 ml	2x 30 ml	- 20°C
Multiplex primer / probe mix	2x 1.5 ml	1x 30 ml	- 20°C
RPP30 Negative Control	1x 0.5 ml	2x 1 ml	- 20°C
2019-nCoV nucleocapsid and envelope genes Positive Control	1x 0.5 ml	2x 1 ml	- 20°C
ROX Reference Dye (10x)	1x 240 µl	2x 1.2 ml	+4°C

Required equipment

- Class II Biological safety cabinet
- Single and/or multichannel pipettes (10, 100, 200, 1000 µl)
- PCR-clean filtered tips
- 1.5 / 2 ml cold block (or access to ice)
- 96 well cold block (or access to ice)
- qPCR Instrument (4 colour)
- 96 well plate and optical seal compatible with qPCR instrument
- 96 well plate compatible vortexer
- 96 well plate compatible minifuge or centrifuge

Additional user supplied consumables

- Molecular biology grade water

Storage and handling

Upon receipt, store all reagents at -20°C.

Thaw the VirPath qRT-PCR Master Mix on ice and keep on ice at all times. Thaw the multiplex Primers / Probes mix at room temperature in the dark and then keep on ice throughout the qRT-PCR setup preparation. If required, remove ROX reference dye (10x) from storage and keep on ice. After thawing, ensure that all reagents are mixed by briefly by vortexing and then spun down. Avoid repeated freeze / thawing whenever possible.

Warning and precautions

This product should be handled only by trained laboratory technicians who are proficient in performing molecular based tests and it should be used in accordance with the principles of good laboratory practice.

Avoid contact with eyes, skin and clothing. Avoid inhalation and ingestion. A copy of the MSDS is available upon request.

Limitations of use

- The procedures in this IFU must be followed as described. Any deviations may result in assay failure or cause erroneous results.
- Good laboratory practice is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded in accordance with local waste disposal regulations.
- Interpretation of results must account for the possibility of false negative and false positive results.
- False negative results may be caused by:
    - Unsuitable collection, handling and/or storage of samples.
    - Failure to follow procedures in this handbook.
- False positive results may be caused by:
    - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
    - Unsuitable handling of amplified product.
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.

Handling of plasmid controls

The RPP30 Negative Control and 2019-nCoV nucleocapsid and envelope genes Positive control consist of plasmids containing the human RPP30 gene; and the nucleocapsid and envelope genes from SARS-CoV-2 respectively and are provided at a concentration of 200 copies/µl. When thawed for the first time, it is recommended to aliquot out the entire amount of both controls in single use aliquots in order to minimize freeze / thaw cycles. For each qRT-PCR run, 5 µl of each control are used (ie 5 µl per reaction = 1000 copies per reaction).

qRT-PCR setup procedure

Table 1. outlines the required volumes of sample / control and reagents needed to set up the qRT-PCR reaction. Each qRT-PCR run should include the No Template Control (ie molecular biology grade water), the RPP30 negative control and the 2019-nCoV nucleocapsid and envelope genes positive control. All procedures should be carried out in a sterile environment, ideally a Class II biosafety cabinet. Thaw reagents as described above and setup the reaction on ice (or cold block). If ROX reference dye is required, follow Table 2 for instruments requiring low ROX and Table 3 for instruments requiring high ROX.

Table 1. qRT-PCR reaction mix setup volumes for a single reaction. When preparing a mix for multiple reactions, include a 5% overage for each reagent.

Reagent	Volume
VirPath qRT-PCR Master Mix	5 µL
Primer and Probe Mix	2.5 µL
Template	10 µL
Water	2.5 µL
Total	**20 µL**

Table 2. qRT-PCR reaction mix setup volumes for a single reaction with low ROX. When preparing a mix for multiple reactions, include a 5% overage for each reagent.

Reagent	Volume
VirPath qRT-PCR Master Mix	5 µL
Primer and Probe Mix	2.5 µL
Template	10 µL
ROX reference dye (10x)	0.2 µL
Water	2.3 µL
Total	**20 µL**

Table 3. qRT-PCR reaction mix setup volumes for a single reaction with high ROX. When preparing a mix for multiple reactions, include a 5% overage for each reagent.

Reagent	Volume
VirPath qRT-PCR Master Mix	5 µL
Primer and Probe Mix	2.5 µL
Template	10 µL
ROX reference dye (10x)	2 µL
Water	0.5 µL
Total	**20 µL**

NOTE: the volume of water and template can be adjusted to include more template in the reaction mix as required.

After setting up the qRT-PCR plate, ensure that the reaction mixtures inside the wells are properly mixed by vortexing the plate and then spinning it down.

qRT-PCR setup and cycling conditions

Set up the qPCR instrument using manufacturers guidelines. Where possible, choose the Quantitation by Comparative Ct (ΔΔCt) method with TaqMan or "Other" reagents (do not add a melt curve option). For qPCR instruments with "FAST" blocks, select the Standard ramp speed. Select the FAM filter for the N1 target, the HEX / VIC filter for the RP target and the Cy5 filter for the E target. If possible, select NFQ-MGB as quencher; or alternatively leave this field empty. Do not select a dye as quencher (such as TAMRA). If required, select ROX as reference dye. Select Program cycling conditions as shown in Table 4 below. Set reaction volume to 20 µl.

Table 4. qRT-PCR Program cycling conditions

Step	Cycles	Temperature	Time
1	1	50 °C	10 min
2	1	95 °C	2 min
3	45	95 °C	5 s
4		62 °C	30 s

NOTE: fluorescence acquisition is performed at step 4.

qRT-PCR data analysis and interpretation

Please note data analysis may vary between qPCR machines and thresholds must be determined empirically by the end user or laboratory. We recommend setting the Baseline start cycle at 5 and the end cycle at 15; and the threshold at 200 RFU or 0.02 ΔCt.

Ct values that fall below the 40 cycles threshold are considered positive signals. Refer to Table 5 below for interpretation of results from control and patient samples.

Table 5. Interpretation of results from control and patient samples

Sample	RP Result	N1 Result	E Result
RPP30 Negative Control	**+**	**-**	**-**
2019-nCoV N / E gene Positive Control	**-**	**+**	**+**
Positive patient sample	**+**	**+**	**+**
	-	**+**	**+**
Negative patient sample	**+**	**-**	**-**
Inconclusive patient sample	**+**	**+**	**-**
	+	**-**	**+**
	-	**+**	**-**
	-	**-**	**+**
Failed patient sample	**-**	**-**	**-**

Kit specification and performance

Application	Qualitative PCR test for detection of SARS-CoV-2 N / E genes
Type of detection	Ribonucleic acid (RNA) of SARS-CoV-2
Sample type	Upper respiratory tract specimens (e.g nasopharyngeal fluids, nasal swab)
qRT-PCR Limit of Detection	$1x10^0$
End-to-end* Limit of Detection (LoD)	750 copies/ml
Analytical specificity *	100%

*VirPath SARS-CoV-2 Multiplex qRT-PCR Kit comprises only primers and probes designed by the CDC from the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel (assays N1 and RP) and by Cortman et al. from the Charité-Berlin WHO protocol (assay E) without any changes. Analytical specificity (cross-reactivity) of these diagnostic panels has been previously established.

qRT-PCR Limit of Detection (LOD) testing

To understand the limit of detection (LOD) for the VirPath Sars-CoV-2 qRT-PCR kit we undertook the following experiment to calculate the minimum copies of viral template RNA which can be detected in the qRT-PCR reaction. Synthetic SARS-CoV-2 RNA: ORF, E, N (ATCC® VR-3276SD™) was used in a serial dilution experiment to establish a limit of detection of VirPath qRT-PCR Master Mix using N1 and E primer and probe mixes on a BioRad CFX96 (Figure 1 and Table 6).

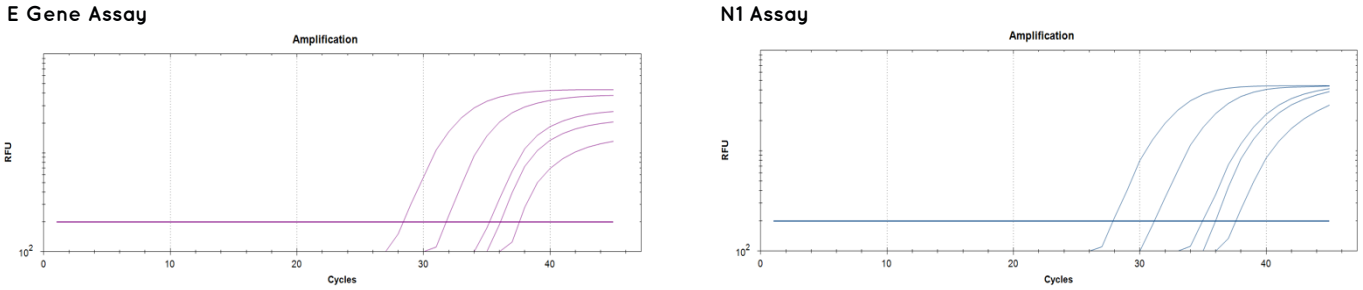


Figure 1. qRT-PCR data of samples containing 1000, 100, 10, 5 and 1 copies of Synthetic SARS-CoV-2 RNA control processed using the VirPath qRT-PCR multiplex kit and run on a BioRad CFX96 qPCR instrument.

Table 6. qRT-PCR replicate and Ct summary data of samples containing 1000, 100, 10, 5 and 1 copies of Synthetic SARS-CoV-2 RNA control extracted using the VirPath Xtract kit, processed using the VirPath qRT-PCR multiplex kit and run on a BioRad CFX96 qPCR instrument.

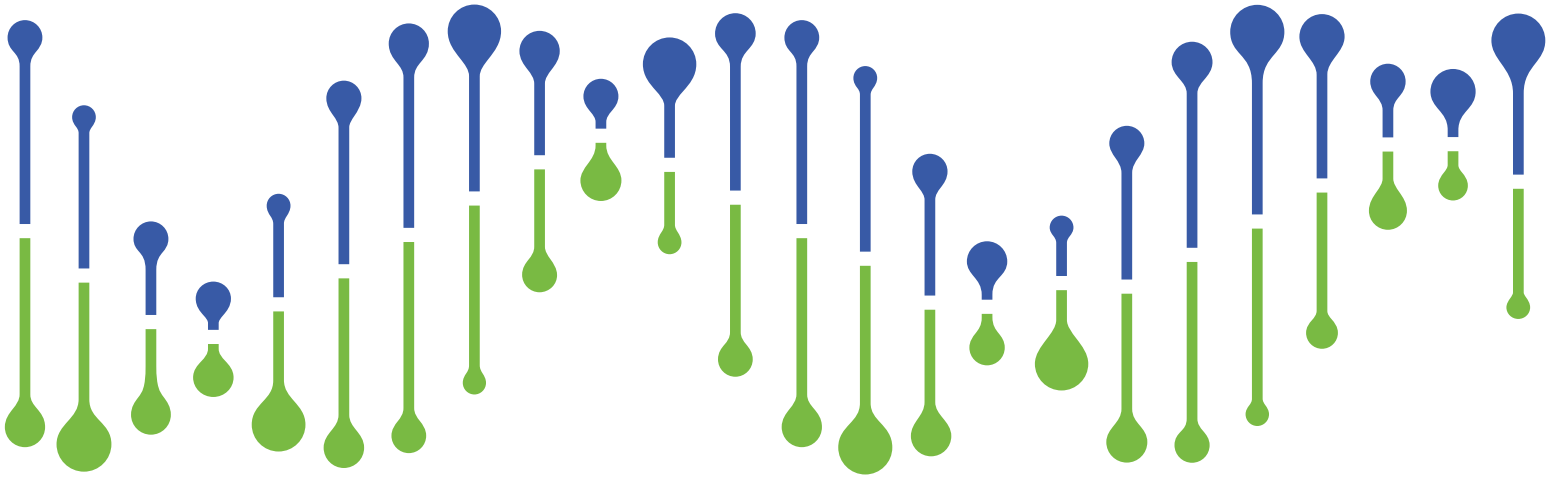
Total copies	Assay E-Sarbeco			Assay N1		
	Replicates	Average Ct	StDev	Replicates	Average Ct	StDev
1000	3/3	28.25	0.10	3/3	27.78	0.14
100	3/3	31.49	0.22	3/3	30.95	0.22
10	3/3	35.12	0.11	3/3	34.48	0.43
5	3/3	36.09	0.42	3/3	35.94	0.77
1	3/3	37.05	0.60	3/3	37.27	0.52
0	0/3	N/A	N/A	0/3	N/A	N/A

End-to-end Limit of Detection (LOD) Testing

In triplicate, an enveloped control sample was spiked into negative swab samples at viral load concentrations equivalent to 250 copies / ml to 100,000 copies / ml. The samples containing the control spike in were extracted using the VirPath Xtract kit, eluted in 100ul of elution buffer and 10ul of the eluate was tested following this protocol (Table 7).

Table 7. qRT-PCR triplicate data of samples containing 250 copies / ml to 100,000 copies of enveloped control sample extracted using the VirPath Xtract kit, processed using the VirPath qRT-PCR kit and run on a BioRad CFX96 qPCR instrument.

Copies / ml	Assay E-Sarbeco			Assay N1		
	Replicates	Average Ct	StDev	Replicates	Average Ct	StDev
250	2/3	35.33341075	0.47	3/3	34.36405619	1.31
500	3/3	35.7605175	0.47	3/3	33.91159677	0.30
750	3/3	34.8828578	0.27	3/3	32.86830049	0.75
1000	3/3	34.80378418	0.50	3/3	32.01858393	0.20
10000	3/3	31.46544347	0.34	3/3	29.06890166	0.35
100000	3/3	28.29607366	0.36	3/3	25.79916957	0.25



www.nonacus.com

Nonacus Limited
Birmingham Research Park, Vincent Drive, Birmingham, B15 2SQ

info@nonacus.com

NONACUS
Advancing Non-Invasive Healthcare