JN Medsys, Revision: 11 Effective date: 28th Mar 2023

ProTect™ COVID-19 RT-qPCR kit 2.0

100 tests (Cat No. 10029)

Instructions for Use

For In-vitro Diagnostic (IVD) Use

IVD

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INTENDED USE

The ProTect™ COVID-19 real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) kit 2.0 is developed for qualitative detection of RNA from the SARS-CoV-2 virus. The ProTect™ COVID-19 RT-qPCR kit 2.0 is validated to detect for SARS-CoV-2 RNA in upper respiratory swab specimens (e.g. nasopharyngeal, oropharyngeal, nasal mid-turbinate, anterior nasal, oropharyngeal + nasal mid-turbinate swabs) collected from suspected infected individuals.

Positive results indicate the presence of SARS-CoV-2 RNA but do not rule out other infections (bacteria and other viruses) and the presence of SARS-CoV-2 RNA may not be the definite cause of disease. Negative results of the SARS-CoV-2 infection should not be used as the sole basis for treatment or other patient management decisions and must be combined with clinical observations, patient history, and epidemiological information.

Testing with the ProTect™ COVID-19 RT-qPCR kit 2.0 is intended for use by trained laboratory personnel who has the proper skills to run RT-qPCR assays.

The ProTect™ COVID-19 RT-qPCR kit 2.0 by JN Medsys provides all necessary reagents for the *in vitro* qualitative detection of SARS-CoV-2 from upper respiratory nasopharyngeal swab specimens and does not include reagents for the extraction and purification of RNA from the SARS-CoV-2 virus. The ProTect™ COVID-19 RT-qPCR kit 2.0 was validated using Applied Biosystems® QuantStudio® 3 and 5 Real-Time PCR Systems and Bio-Rad CFX96 Dx Real-Time PCR Detection System. The test is compatible with the US CDC protocol, targeting SARS-CoV-2 N1 and N2 genes and the human RNase P control gene.

SUMMARY AND EXPLANATION

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus now called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; formerly called 2019-nCoV).

SARS-CoV-2, which is the causative agent of the pneumonia outbreak in Wuhan City, Hubei Province, China, was reported to World Health Organization (WHO) on December 31, 2019. This novel coronavirus was later identified, although it had already resulted in thousands of confirmed human infections in multiple provinces throughout China and many countries subsequently including Singapore. SARS-CoV-2 is known to be capable of asymptomatic infection, mild illness, severe illness, and cause death.

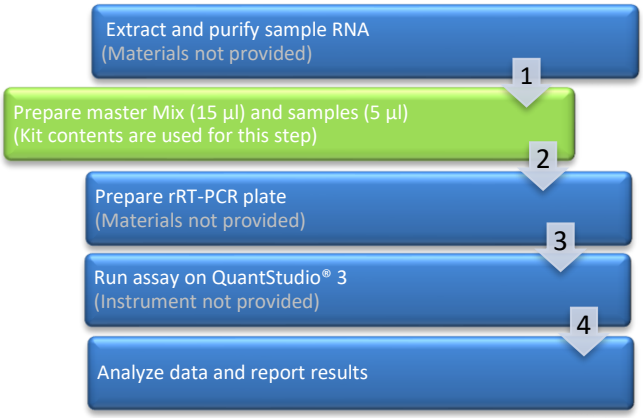
The ProTect™ COVID-19 RT-qPCR kit 2.0 is a molecular *in vitro* test that detects for SARS-CoV-2 viral RNA. The detection of the viral RNA will aid in the diagnosis of SARS-CoV-2 and is based on RT-qPCR technology. The product contains oligonucleotide primers and dual-labelled hydrolysis probes (TaqMan®) and control materials used in RT-qPCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in upper respiratory swab specimens.

PRINCIPLES OF THE PROCEDURE

The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected from regions of the virus nucleocapsid (N) gene. The kit is designed for the specific detection of 2 regions on the SARS-CoV-2 (two primer/probe sets) N gene. An additional primer/probe set is also included in the kit to detect for the human RNase P gene (RP) in control samples and clinical specimens.

The viral RNA is first extracted and purified from upper respiratory swab specimens using nasal swabs or oropharyngeal specimens using throat swabs. The purified RNA is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems® QuantStudio® 3 Real-Time PCR System. In the process, the probe first anneals to its specific target sequence by base pairing and designed to be located in the region between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe which is already bound to the specific sequence, causing the reporter dye to separate from the quencher dye, resulting in a fluorescent signal. With each amplification cycle, additional reporter dye molecules are liberated, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle and will result in a cycle threshold (ct) value. This ct value will be used to determine whether the target is present or not.

Summary of testing process



KITS AND EQUIPMENT USED

The following kits and equipment were used in the validation of ProTect™ COVID-19 RT-qPCR kit 2.0:

Items	Details
Nasopharyngeal nasal swabs	Copan 3ml Universal Transport Medium™ (Cat No: 305C)
Viral RNA extraction and purification	Liferiver™ EX3600 Automated Nucleic Acid Extraction System PerkinElmer Nucleic Acid Extraction Kit (Cat No: SY609)
qPCR instrument	Applied Biosystems® QuantStudio® 3 and 5 Real-Time PCR Systems Bio-Rad CFX96 Dx Real-Time PCR Detection System

The above table is for reference purposes only and users are recommended to validate the kits and equipment used (including those listed above) with ProTect™ COVID-19 RT-qPCR kit 2.0.

WARNINGS AND PRECAUTIONS

- Patient specimens and positive controls should assumed to be potentially infectious and handled properly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Maintain separate areas for assay setup and handling of nucleic acids.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.

- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Always check the expiration date and do not use expired reagents.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Use and change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
    - Allocate separate equipment and supplies for assay setup and for handling extracted RNA.
    - Wear a clean lab coat and new powder-free disposable gloves when setting up assays.
    - Gloves should be changed between samples or whenever contamination is suspected.
    - Reagents and reaction tubes should be capped or covered as much as possible.
    - Primers, probes, and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.
    - Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products (e.g., 10% bleach, “DNAZap™” or “RNase AWAY®”) before every test to minimize risk of nucleic acid contamination.
    - RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
    - Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

SPECIMEN COLLECTION, HANDLING, AND STORAGE (MATERIALS NOT PROVIDED)

- Improper specimen collection, storage, and/or transport are likely to affect test results. Specimens should be collected by trained personnel due to the importance of specimen quality.

Collecting the Specimen

- Follow manufacturer instructions on specimen collection device for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip. Do not use Calcium alginate swabs and cotton swabs with wooden shafts are not recommended. After collection, place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

Storing Specimens

- Specimens should be stored following the manufacturer recommendation and/or laboratory's standard procedures.

EXTRACTION PROCEDURE

Performance of the ProTect™ COVID-19 RT-qPCR kit 2.0 is dependent on the quantity and quality of template viral RNA purified from human upper respiratory swab specimens. The Liferiver™ EX3600 Automated Nucleic Acid Extraction System and PerkinElmer Nucleic Acid Extraction Kit (Cat No: SY609) were used to extract viral RNA from specimens and validated for use with this kit. For kits not listed here, it is recommended for users to validate them for use with ProTect™ COVID-19 RT-qPCR kit 2.0.

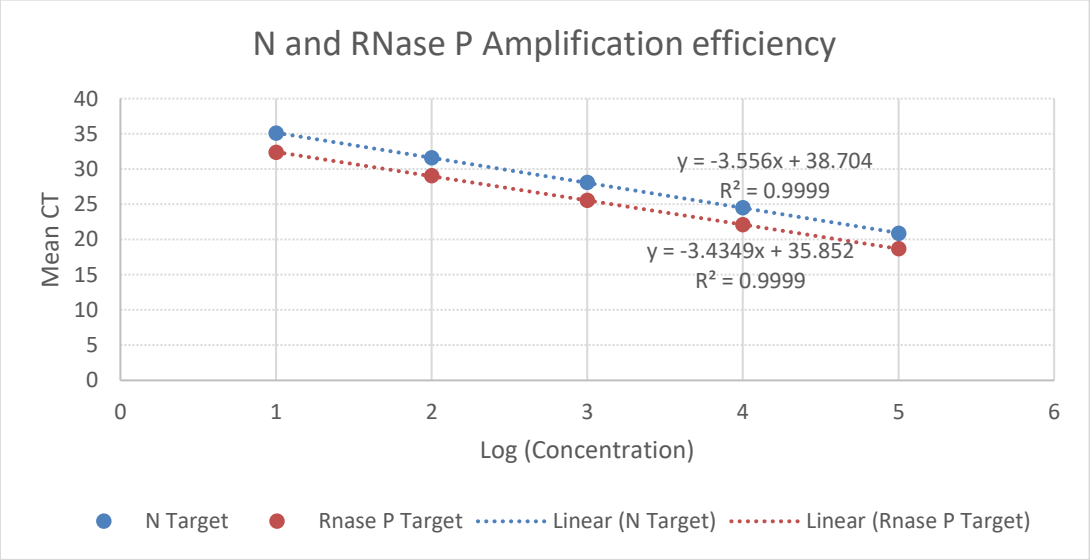
KIT FEATURES

Test Principle	One-step RT-qPCR (TaqMan®-based detection)
Targets	N1, N2 (SARS-CoV-2, FAM) and RNase P (Human, HEX)
Number of Tests	100/kit
Compatible Specimen Type	Upper respiratory nasopharyngeal swab specimens (i.e. nasopharyngeal, oropharyngeal, nasal mid-turbinate, anterior nasal, oropharyngeal + nasal mid-turbinate swabs)
Limit of Detection	5 copies RNA per reaction
Amplification efficiency	N >90%, RNase P > 95%
Precision	<2%
Specificity	Detects only SARS-CoV-2 based on *in silico* sequence validation and wet lab cross-reactivity testing

PERFORMANCE CHARACTERISTICS

Amplification efficiency

A series of 10-fold serial dilutions was prepared and analysed in duplicates with the ProTect™ COVID-19 RT-qPCR Kit 2.0. A standard curve is subsequently generated based on the mean CT values for each serial dilution to determine the amplification efficiency of the assay.



Target	Slope	Efficiency
N	-3.56	91.08%
RNase P	-3.43	95.49%

Amplification efficiencies for both N target and RNase P target are above 90%.

Limit of Detection (LoD)

The stock concentration (copies/ul) of AMPLIRUN® SARS-CoV-2 RNA control was first quantified using the Clarity™ Digital PCR System (JN Medsys; Cat number: 10001). Four 2-fold serial dilutions (10, 5, 2.5, and 1.25 copies/reaction) were subsequently prepared from the stock solution and tested using the ProTect™ COVID-19 RT-qPCR Kit 2.0 to determine the preliminary LoD on the Applied Biosystems® QuantStudio® 5 (QS5) and QuantStudio® 3 (QS5) Real-Time PCR Systems. The preliminary LoD was defined as the lowest viral RNA concentration having all three replicates tested positive (100% positivity).

The LoD confirmatory test was performed by testing 20 replicates of the control RNA at (i) preliminary LoD concentration and (ii) 2-fold diluted concentration of (i). The LoD is defined as the lowest concentration at which ≥ 95 % (19/20) of the tested replicates are positive.

Preliminary LoD test on QS5

Target	Concentration (Copies/Reaction)	Number of Replicates Tested Positive (out of 3)	CT	Mean CT	Standard Deviation	RU (%)
N	10	3	34.86	35.05	0.39	1.12%
			35.50			
			34.79			
	5	3	34.30	33.83	1.48	4.36%
			35.02			
			32.18			
	2.5	1	Undetermined	-	-	-
			35.42			
			Undetermined			
	1.25	0	Undetermined	-	-	-
			Undetermined			
			Undetermined			

LoD confirmation test on QS5

Reaction No.	Target Name	CT	Total positive detection (out of 20)	Target Name	CT	Total positive detection (out of 20)
1	**N 5 copies/ reaction**	Undetermined	19	**N 2.5 copies/ reaction**	35.63	9
2		33.73			29.62	
3		34.43			Undetermined	
4		34.83			32.71	
5		33.84			34.09	
6		31.70			Undetermined	
7		36.26			35.74	
8		35.30			34.68	
9		33.93			Undetermined	
10		31.96			33.24	
11		33.29			35.33	
12		33.94			33.64	
13		33.57			35.31	
14		34.04			Undetermined	
15		35.42			Undetermined	
16		33.47			Undetermined	
17		32.99			33.83	
18		34.85			Undetermined	
19		33.02			30.06	
20		30.77			34.15	

Preliminary LoD test on QS3

Target	Concentration (Copies/Reaction)	Number of Replicates Tested Positive (out of 3)	CT	Mean CT	Standard Deviation	RU (%)
N	5	3	35.00	34.32	0.60	1.74%
			34.02			
			33.93			
	2.5	3	35.75	35.66	0.08	0.22%
			35.65			
			35.59			
	1.25	1	Undetermined	-	-	-
			Undetermined			
			35.47			

LoD confirmation test on QS3

Reaction No.	Target Name	CT	Total positive detection (out of 20)	Target Name	CT	Total positive detection (out of 20)
1	**N 5 copies/ reaction**	36.99	20.00	**N 2.5 copies/ reaction**	Undetermined	15
2		34.07			Undetermined	
3		34.69			35.04	
4		33.63			34.30	
5		33.78			34.43	
6		34.77			Undetermined	
7		34.67			35.83	
8		33.36			34.52	
9		34.09			35.62	
10		33.94			Undetermined	
11		33.89			36.02	
12		34.89			34.87	
13		34.80			34.21	
14		33.41			35.47	
15		34.61			34.60	
16		34.64			35.47	
17		33.47			34.62	
18		34.03			35.34	
19		33.79			34.79	
20		33.67			Undetermined	

LoD test was performed to determine the lowest detectable concentration of SARS-CoV-2 RNA using both the Applied Biosystems® QS5 and QS3 qPCR systems.

For QS5, the preliminary LoD was determined to be 5 copies/reaction where 3 out of 3 replicates were tested positive. The confirmatory LoD was also determined to be 5 copies/reaction where 95% of the replicates were tested positive.

For QS3, the preliminary LoD was determined to be 2.5 copies/reaction where 3 out of 3 replicates were tested positive. However, when 20 replicates of 2.5 copies/reaction were analyzed, only 15 out of 20 replicates were positive (75% positivity). Hence, the confirmatory LoD test was repeated at a 2-fold more concentrated sample (i.e. 5 copies/reaction) where 20 out of 20 samples were positive. Hence, the LoD was determined to be 5 copies/reaction.

The LoD of ProTectTM COVID-19 RT-qPCR Kit 2.0 is determined to be 5 copies/reaction for both QS3 and QS5.

Precision

Precision evaluation (repeatability and reproducibility) was conducted at JN Medsys using the QS5 qPCR system. The 20X2X2 study, which involves performing the test for 20 days with 2 runs per day, and two replicates per run, was performed to evaluate repeatability. 2 samples were evaluated: (1) Positive control from ProTect Kit 2.0 and (2) a 5XLoD sample. Reproducibility study involving 3 operators, 5 different lots of ProTect Kit 2.0 and 5 replicates of the kit's positive control per run were also conducted.

(A) Repeatability results

Run	Sample 1 - Positive Control		Sample 2 - 5X LoD	
	Mean CT		Mean CT	
	N (FAM)	RNase P (HEX)	N (FAM)	RNase P (HEX)
Day 1-1	22.28	23.45	32.17	31.73
Day 1-2	22.35	23.58	32.08	31.49
Day 2-1	22.83	23.67	32.03	31.01
Day 2-2	22.79	23.54	32.11	31.03
Day 3-1	22.61	23.86	33.08	31.48
Day 3-2	23.19	24.00	31.87	30.96
Day 4-1	23.15	24.03	32.29	31.61
Day 4-2	22.70	23.62	32.16	31.34
Day 5-1	22.40	23.59	32.08	31.46
Day 5-2	22.96	23.97	32.37	31.34
Day 6-1	22.84	23.63	32.28	31.17
Day 6-2	22.90	23.58	32.34	31.36
Day 7-1	22.70	23.50	31.94	30.88
Day 7-2	22.79	23.67	31.91	31.13
Day 8-1	22.91	23.74	32.07	30.95
Day 8-2	22.63	23.50	31.96	30.75
Day 9-1	22.74	23.65	31.62	30.72
Day 9-2	22.89	23.58	31.96	31.02
Day 10-1	23.02	23.83	32.02	31.02
Day 10-2	22.43	23.60	32.14	31.21
Day 11-1	23.77	24.65	32.18	31.39
Day 11-2	23.14	24.17	32.38	30.99
Day 12-1	22.35	23.63	32.53	32.01
Day 12-2	22.85	23.80	32.02	31.33
Day 13-1	22.93	23.81	32.22	31.34
Day 13-2	22.83	23.96	32.44	31.82
Day 14-1	22.77	23.64	32.11	31.24
Day 14-2	22.69	23.69	31.75	31.05
Day 15-1	22.97	23.85	32.41	31.78

Day 15-2	22.85	23.74	31.99	31.21
Day 16-1	22.94	23.80	32.25	31.17
Day 16-2	22.92	23.85	32.11	31.22
Day 17-1	22.81	23.82	32.38	31.34
Day 17-2	22.94	23.80	32.21	31.09
Day 18-1	22.65	23.30	32.28	31.27
Day 18-2	23.02	24.07	32.22	31.18
Day 19-1	22.48	23.52	32.22	31.03
Day 19-2	22.43	23.72	31.73	31.25
Day 20-1	22.57	23.72	31.93	31.07
Day 20-2	22.81	23.69	32.18	30.94
Overall Mean	**22.80**	**23.74**	**32.15**	**31.23**
Standard Deviation	**0.28**	**0.23**	**0.25**	**0.28**
Relative Uncertainty (%)	**1.22**	**0.98**	**0.78**	**0.91**

(B) Reproducibility results

	Operator 1		Operator 2		Operator 3				
Lot No.	**N (FAM)**	**RNase P (HEX)**	**N (FAM)**	**RNase P (HEX)**	**N (FAM)**	**RNase P (HEX)**			
Lot1	22.62	23.61	22.63	23.61	22.63	23.48			
	22.51	23.51	22.61	23.65	22.60	23.40			
	22.55	23.59	22.57	23.61	22.63	23.41			
	22.58	23.62	22.63	23.60	22.63	23.48			
	22.60	23.67	22.63	23.56	22.48	23.35			
Lot2	21.50	23.54	21.96	23.60	22.52	23.81			
	21.98	23.75	22.03	23.64	22.54	23.80			
	21.89	23.66	22.10	23.59	22.46	23.69			
	21.85	23.61	21.98	23.61	22.46	23.82			
	21.44	23.48	22.05	23.68	22.01	23.66			
Lot3	22.58	23.64	22.96	23.99	22.80	23.71			
	22.47	23.52	22.91	23.91	22.81	23.74			
	22.53	23.57	22.94	23.94	22.80	23.74			
	22.52	23.60	22.90	23.96	22.73	23.67			
	22.60	23.67	23.00	24.10	22.83	23.75			
Lot4	22.52	23.81	22.53	23.69	22.52	23.73			
	22.49	23.77	22.79	23.80	22.46	23.70			
	22.53	23.80	22.64	23.77	22.63	23.79			
	22.53	23.85	22.70	23.78	22.66	23.78			
	22.59	23.83	22.77	23.83	22.61	23.80			
Lot5	22.36	23.71	21.97	23.41	22.41	23.52			
	22.34	23.74	21.98	23.49	22.41	23.53			
	22.35	23.67	22.00	23.48	22.42	23.44			
	22.44	23.79	22.03	23.54	22.45	23.63			
	22.38	23.75	21.97	23.53	22.41	23.52	**Overall**	**N (FAM)**	**RNase P (HEX)**
Mean	**22.35**	**23.67**	**22.45**	**23.69**	**22.56**	**23.64**	**Mean**	22.45	23.67
SD	**0.34**	**0.11**	**0.39**	**0.18**	**0.18**	**0.15**	**SD**	0.32	0.15
RU (%)	**1.53**	**0.45**	**1.75**	**0.76**	**0.79**	**0.63**	**RU (%)**	**1.44**	**0.63**

Based on the repeatability and reproducibility studies, the ProTect™ COVID-19 RT-qPCR Kit 2.0 can achieve a relative uncertainty of up to 1.75% (<2%), hence demonstrating the high precision of the kit.

Inclusivity

SARS-CoV-2 genome sequences uploaded onto GISAID from 1st Oct 2020 to 30th Sep 2022 were retrieved for the analysis. The sequences were then analysed for the presence of mutations in the primer and/or probe regions for both N1 and N2. Wet lab testing was also performed on sequences containing both N1 and N2 mutations to ascertain if the kit remains effective in detecting these mutants.

Period	Total sequences analysed	Number of sequences containing mutations on both N1 and N2, and not detectable by kit	% Sensitivity
1st Oct-31st Dec 2020	141514	0	100%
1st Jan-31st Jan 2021	110821	0	100%
1st Feb-28th Feb 2021	132847	0	100%
1st Mar-30th Apr 2021	503089	0	100%
1st May-31st May 2021	344446	0	100%
1st Jun - 30th Jun 2021	245902	0	100%
1st Jul - 31st Jul 2021	265189	0	100%
1st Aug-31st Aug 2021	453351	0	100%
1st Sep-30th Sep 2021	533004	0	100%
1st Oct-31st Oct 2021	575643	0	100%
1st Nov-30th Nov 2021	588915	0	100%
1st Dec-31st Dec 2021	652110	0	100%
1st Jan – 31st Jan 2022	202394	0	100%
1st Feb - 28th Feb	124399	0	100%
1st Mar – 31st Mar	134246	0	100%
1st Apr-30th Apr 2022	139803	0	100%
1st May-31st May 2022	92000	0	100%
1st June-30th June 2022	62638	0	100%
1st July-31st July 2022	59120	0	100%
1st Aug -31st Aug 2022	26942	0	100%
1st Sep-30th Sep 2022	14889	0	100%
Total	**5403262**	**0**	**100%**

From the in-silico sequence analysis and wet lab testing, ProTect™ COVID-19 RT-qPCR kit 2.0 is able to detect all known SARS-CoV-2 strains from uploaded to GISAID from 1st Oct 2020 to 30th Sep 2022.

Analytical Specificity

(A) Wet testing cross-reactivity

The ProTect™ COVID-19 RT-qPCR Kit 2.0 was evaluated for cross-reactivity with other human coronaviruses and common respiratory pathogens on the QS5 qPCR system. Nucleic acids from intact inactivated viruses [(229E, OC43, NL63 and HKU1; Zeptometrix) & (AMPLIRUN® Total Respiratory Viral Panel Control; Vircell)] were first extracted using the EX3600 Automated Nucleic Acid Extraction System (Liferiver™) prior to analysis. On the other hand, purified RNAs (MERS, SARS-CoV-1, SARS-CoV-2; Vircell) were used in the evaluation directly without any processing.

Human coronaviruses tested in cross-reactivity study

No.	Human coronaviruses	Product name	Manufacturer	Testing concentration
1	Human coronavirus 229E	NATtrolTM Respiratory Pathogen Panel-1	Zeptometrix	Ct 25-28^
2	Human coronavirus OC43	NATtrolTM Respiratory Pathogen Panel-1	Zeptometrix	Ct 25-28^
3	Human coronavirus NL63	NATtrolTM Respiratory Pathogen Panel-1	Zeptometrix	Ct 25-28^
4	Human coronavirus HKU1	NATtrolTM Respiratory Pathogen Panel-1	Zeptometrix	Ct 25-28^
5	MERS-coronavirus	AMPLIRUN® MERS CORONAVIRUS RNA CONTROL	Vircell	12500- 20000 copies/µl
6	SARS-coronavirus-1	AMPLIRUN® CORONAVIRUS SARS (2003) RNA CONTROL	Vircell	12500- 20000 copies/µl
7	SARS-coronavirus-2	AMPLIRUN® SARS-CoV-2 RNA CONTROL	Vircell	12500- 20000 copies/µl

^ Specifications of the viruses were provided by the manufacturer in terms of CT values instead of viral copies.

Common respiratory pathogens tested in cross-reactivity study

No.	Human respiratory pathgens	Product name	Manufacturer	Testing concentration
1	ADENOVIRUS 4	AMPLIRUN® Total Respiratory Viral Panel Control	Vircell	20-50 copies/ µl
2	CORONAVIRUS			
3	INFLUENZA A H3N2			
4	INFLUENZA B			
5	NOVEL INFLUENZA A H1N1			
6	PARAINFLUENZA 1			
7	PARAINFLUENZA 2			
8	PARAINFLUENZA 3			
9	RESPIRATORY SYNCYTIAL VIRUS (subtype A)			
10	RESPIRATORY SYNCYTIAL VIRUS (subtype B)			

(ii) Results

Results for Wet testing cross-reactivity are summarized below:

Wet testing cross-reactivity result for human coronavirus

Target	Human coronavirus	CT1	CT2	Mean CT
N	229E (Zeptometrix)	Undetermined	Undetermined	Undetermined
	OC43 (Zeptometrix)	Undetermined	Undetermined	Undetermined
	NL63 (Zeptometrix)	Undetermined	Undetermined	Undetermined
	HKU1 (Zeptometrix)	Undetermined	Undetermined	Undetermined
	MERS (Vircell)	Undetermined	Undetermined	Undetermined
	SARS-CoV-1 (Vircell)	Undetermined	Undetermined	Undetermined
	SARS-CoV-2 (Vircell)	30.63	30.42	30.53
	Positive Control (ProTect 2.0)	22.79	22.63	22.71
	NTC	Undetermined	Undetermined	Undetermined

Wet testing cross-reactivity result for common respiratory pathogens

Target	Respiratory pathogens	CT1	CT2	Mean CT
N	AMPLIRUN® Total Respiratory Viral Panel Control	Undetermined	Undetermined	Undetermined
	Positive Control (ProTect 2.0)	24.20	25.04	24.62
	NTC	Undetermined	Undetermined	Undetermined

(B) In silico cross-reactivity

In silico analysis was also performed to evaluate the potential cross-reactivity between ProTect™ COVID-19 RT-qPCR Kit 2.0 primers and probes and other organisms. BLASTN were conducted with the exclusion of SARS-CoV-2 (taxid:2697049), synthetic constructs (taxid:32630) and cloning vectors (taxid:29278) from the analysis. The results shown that ProTect™ COVID-19 RT-qPCR Kit 2.0 primers and probes does not show any cross-reactivity with non-target pathogens.

Interference

The ProTect™ COVID-19 RT-qPCR Kit 2.0 was evaluated for interference arising from mucin and blood. Three conditions and their controls (contrived positive, contrived negative, no template control) were evaluated as illustrated in the table below. Blood and/or mucin was first spiked into UTM containing upper nasopharyngeal swab sample accordingly. The spiked UTM was then extracted using the EX3600 Automated Nucleic Acid Extraction System (Liferiver™) and the purified RNA (5x LoD concentration; Twist) was used in this evaluation.

Results

Result for the interference evaluation of mucin and blood are detailed below.

Interference results

Interference Study			
	N target		
Spike Conditions	**Ct**	**Average Ct**	**RU(%)**
Contrived positive + Blood 10% v/v	31.61	31.52	0.37
	31.44		
Contrived positive + 50 mg/mL Mucin	32.35	32.57	0.96
	32.79		
Contrived positive + Blood 5% v/v + Mucin 25mg/ml	32.17	32.26	0.40
	32.35		
Contrived positive	31.04	30.90	0.65
	30.76		
Contrived negative	Undetermined	NA	NA
	Undetermined		
NTC	Undetermined	NA	NA
	Undetermined		

The ProTect™ COVID-19 RT-qPCR Kit 2.0 was able to positively detect for 5x LoD of Twist controls despite the presence of spiked blood and/or mucin. Ct values of blood and/or mucin spiked samples show a slight delay in CT value but remains detectable by the kit. Based on the results, the ProTect™ COVID-19 RT-qPCR Kit 2.0 can tolerate these interferences.

Clinical Evaluation

The clinical performance of the ProTect™ COVID-19 RT-qPCR Kit 2.0 kit was validated by two 3rd party clinical laboratories. This evaluation was conducted using viral RNA samples extracted from 815 nasopharyngeal swab specimens (combination of prospective and retrospectively collected samples; extracted using EX3600 Automated Nucleic Acid Extraction System or PerkinElmer Nucleic Acid Extraction Kit) whose clinical status were determined using either the PerkinElmer SARS-CoV-2 Real-time RT-PCR Assay or the VereRT™ COVID-19 PCR Kit. The results are summarized in the following table:

		Comparator Kit		
		Positive	**Negative**	**Total**
ProTect™ COVID-19 RT-qPCR Kit 2.0	**Positive**	**296**	**3**	**299**
	Negative	**8**	**508**	**516**
	Total	**304**	**511**	
		Sensitivity (%)	**Specificity (%)**	
		97.4	99.4	

Based on the above data, the ProTect™ COVID-19 RT-qPCR Kit 2.0 kit showed good concordance with the comparator kit demonstrating 97.4% sensitivity and 99.4% specificity.

KIT CONTENTS

Each kit includes reagents sufficient to perform 100 tests (Cat No. 10029). Each test include 1 duplex RT-qPCR assays, which target the N1, N2 on FAM, and RNase P on Hex.

Reagents Supplied	Tube Cap	100 Tests (10029) Volume (µL)
ProTect™ Probe qPCR Mastermix (2X)	Red-labelled	1 x 1000
ProTect™ RT Mix (50X)	Green-labelled	1 x 45
ProTect™ Primer & Probe Mix	Purple-labelled	1 x 510
ProTect™ Positive Control^*	Yellow-labelled	1 x 110
Nuclease Free Water	Blue cap	1 x 2000

^Sufficient for 20 runs

** The ProTect™ Positive Control is made up of plasmid consisting of N1, N2 and RNase P sequences. This serves as a control for the N1, N2 and RNase P tests.*

STORAGE AND STABILITY

The ProTect™ COVID-19 RT-qPCR kit 2.0 should be stored at -20°C upon receipt. Avoid repeated freezing and thawing of kit contents. The kit is stable through the expiry date indicated on the kit label (25 months shelf life).

ASSAY SETUP

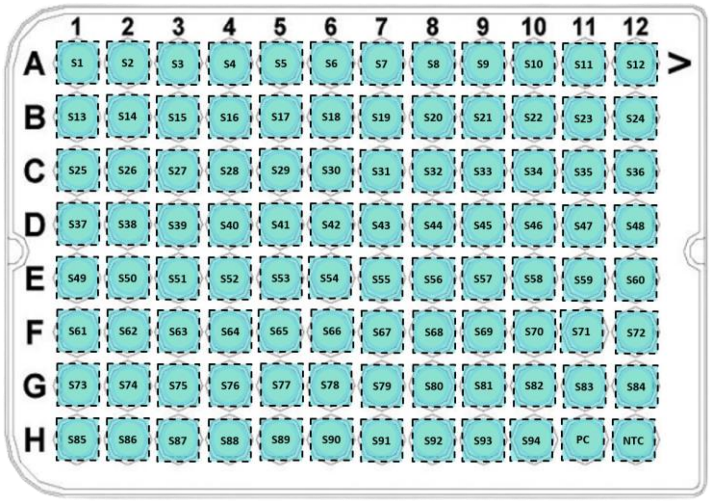
1. Thaw reagents at room temperature and maintain reagents on ice when thawed. Mix reagents gently and briefly centrifuge to collect contents at the bottom of the tubes.
2. Prepare each reaction mix as shown in the table below:

No.	Reagents	Volume (µL)
1	ProTect™ Probe qPCR Mastermix (2X) [Red-labelled]	10
2	ProTect™ RT Mix (50X) [Green-labelled]	0.4
3	ProTect™ Primer & Probe Mix [Purple-labelled]	4.6
4	RNA Sample/ ProTect™ Positive Control [Yellow-labelled] / Water [Blue cap] (NTC)	5*
	Total Vol	20

❗ Positive and no template controls should be included in each run

* Added straight into wells containing 15 µL of the reaction mix

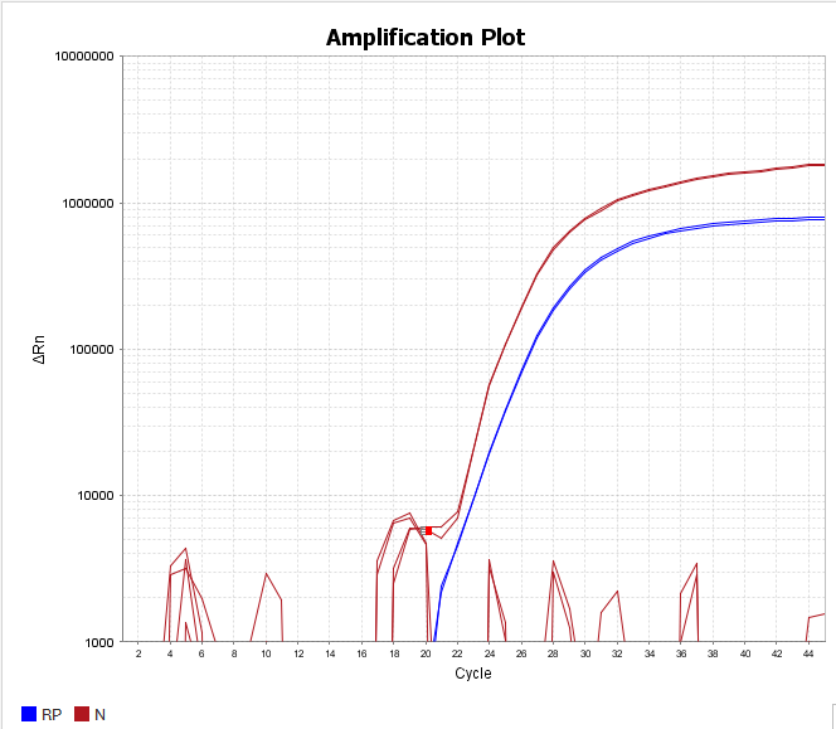
3. Pipette 15 µL of the reaction mix into the required reaction tube strip or 96-well plate. (Table below shows an example of run setup) and add 5 µL of the sample



NTC: No Template Control
S: Samples
PC: Positive Control

4. Centrifuge to collect contents at the bottom of the tube strip/plate.
5. Transfer tube strip/plate to qPCR instrument
6. For QuantStudio® 3 Real-Time PCR System, refer to user manual for machine operation and experimental setup (https://www.thermofisher.com/order/catalog/product/A28567#/A28567). *Do not set reference dye setting. The kit **does not** contain reference dye (e.g. ROX).
7. Perform one-step RT-qPCR according to the following protocol. Fluorescence data for FAM and HEX should be collected during the 55°C annealing & extension step.
8. Analyze results from the plot. PC curves should be smooth and NTC should not result in any ct values.

Step	Cycle	Temperature	Time
Reverse Transcription	1	45°C	15 min
Reverse Transcriptase Inactivation & DNA Polymerase Activation	1	95°C	2 min
Denaturation	40	95°C	3 sec
Annealing & Extension		55°C	30 sec



DATA ANALYSIS AND INTERPRETATION

Interpretation of ProTect™ COVID-19 RT-qPCR kit 2.0 test result should take into consideration the CT values, as well as the shape of the amplification curve.

Extraction and Positive Control Results and Interpretation

1. No Template Control (NTC)

   The NTC consists of using nuclease-free water in the RT-qPCR reactions instead of RNA sample. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and use new reagents. Repeat the assay with strict adherence to the guidelines.

2. SARS-CoV-2 Positive Control (PC)

   The PC consists of plasmid consisting of N1 and N2 target sequences and plasmid containing RNase P sequence. The PC will yield a positive result with the following primer and probe sets: ProTect™ Primer & Probe Mix

3. RNase P (Extraction Control)

   All clinical samples is expected to contain the RNase P RNA and should have a ct value of less than 40 cycles (< 40 ct). If RNase P is not detected in any clinical specimens, it may be due to:

   1. Improper collection resulting in loss of specimen integrity
   2. Improper storage before extraction
   3. Improper extraction of nucleic acid from clinical samples
   4. Improper assay set up and execution.
   5. Improper use of reagents or equipment malfunction.

   If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

   − If the SARS-CoV-2 N are positive and RP is negative, the result should be considered valid. A negative RP signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.

   − If all SARS-CoV-2 N target AND RP are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

4. SARS-CoV-2 Marker (N)

   When all controls exhibit the expected performance, a specimen is considered positive if N cycle threshold is less than 40 cycles (< 40 ct). The RNase P may or may not be positive as described above, but the SARS-CoV-2 result is still valid.

   When all controls exhibit the expected performance and the SARS-CoV-2 N AND the RNase P marker are more than 40 cycles (> 40 ct), the result is invalid. The test should be re-run:

   1. The extracted RNA from the specimen should be re-tested.
   2. If residual RNA is not available, re-extract RNA from residual specimen and re-test.
   3. If the re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.

   When all controls exhibit the expected performance, a specimen is negative for SARS-CoV-2 when SARS-CoV-2 target N cycle threshold are more than 40.00 cycles (> 40) and the cycle threshold of RP is less than 40 cycles (< 40 ct).

Summary

1. No Template Control - No fluorescence signal should be detected
2. Positive Control – Fluorescence signal should be detected with Ct value below 30
3. Results for the respective targets may be interpreted as follow:

N (FAM)	RNase P (HEX)	Outcome
+	+	SARS-CoV-2 detected
-	+	SARS-CoV-2 not detected
-	-	Invalid result. Repeat test

QUALITY CONTROL

a. Quality control procedures are in place for reagent monitoring and to inspect assay performance.

b. Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.

c. A positive extraction control is recommended to be included in each nucleic acid isolation batch in concordance with Good laboratory practice (cGLP)

d. Always include a negative control (NTC), and the appropriate positive control provided (PC) in each amplification and detection run. All clinical samples should be tested for human RNase P gene for interpretations listed above

LIMITATIONS

• The kit is intended to be used by trained personnel as this procedure requires technical skills to perform. They should be able to perform the test and interpret the results independently.

• Performance of the ProTect™ COVID-19 RT-qPCR kit 2.0 has only been established in upper respiratory swab specimens (nasopharyngeal, oropharyngeal, nasal mid-turbinate, anterior nasal, oropharyngeal + nasal mid-turbinate swabs).

• Negative results should not be used as the sole basis for treatment or other patient management decisions.

• False negative results may occur if qPCR inhibitors are present in the sample.

• Do not use any reagent past the expiration date.

• If the virus mutates in the RT-qPCR target region, performance of the kit may be affected

• Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.

• The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.

• The performance of this test has not been established for screening of lower respiratory, blood or blood products for the presence of SARS-CoV-2.

• This test cannot determine diseases caused by other bacterial or viral pathogens.

Revision History

Revision	Effective Date	Description of Change
01	30 June 2020	1. Initial release for use
02	20 July 2020	1. Included CE mark and EU Rep Details
03	01 Dec 2020	1. Included colour codes for reagents tubes (pages 10 & 11) 2. Included page numbers 3. Removed "For Research Use" on page 5
04	05 Oct 2021	1. Included oropharyngeal clinical matrix
05	03 Nov 2021	1. Included nasal mid-turbinate, anterior nasal, oropharyngeal + nasal mid-turbinate clinical matrix
06	07 Jul 2022	1. Uprevision of the footer and added document number
07	04 Aug 2022	1. Updated performance data
08	21 Oct 2022	1. Removed "This assay has received PSAR Authorisation from the Health Sciences Authority in Singapore" from cover page and page3. 2. Updated recommended kits and equipment (pg 5) 3. Updated specimen storage recommendation (pg 7) 4. Updated Inclusivity data to Sept 2022 (pg 14) 5. Updated comparator kit information under "Clinical Evaluation" (pg 18) 6. Updated kit shelf life to 25 months (pg 19)
09	09 Nov 2022	1. Updated information on Kits and Equipment used (pg 5, 18) 2. Updated information on test viruses (pg 15)
10	21 Dec 2022	1. Changed contact email to info@jnmedsys.com 2. Changed "upper respiratory specimens" to "upper respiratory swab specimens" 3. Included reproducibility data in Precision section
11	30 Mar 2023	1. Remove CE-marking and Obelis Information