JN Medsys, Revision: 02 Effective date: 07 Jul 2022

ProTect™ Omivar RT-qPCR kit

100 tests (Cat No. 10035)

Instructions for Use

For Research Use Only (RUO)

Contact Details

JN Medsys Pte Ltd
217 Henderson Road #02-08
Singapore 159555

techsupport@jnmedsys.com | +65 6273 7647 | www.jnmedsys.com

Contents

INTENDED USE 3
SUMMARY AND EXPLANATION 4
PRINCIPLES OF THE PROCEDURE 4
Summary of testing process 5
RECOMMENDED EQUIPMENT 5
WARNINGS AND PRECAUTIONS 5
KIT FEATURES 7
PERFORMANCE CHARACTERISTICS 7
Amplification efficiency: 7
Limit of Detection (LoD): 8
Specificity 8
IN SILICO ANALYSIS OF PRIMER AND PROBE SEQUENCES 9
KIT CONTENTS 9
STORAGE AND STABILITY 9
ASSAY SETUP 10
DATA ANALYSIS AND INTERPRETATION 12
Interpretation of ProTect™ Omivar RT-qPCR kit test result should take into consideration the CT values, as well as the shape of the amplification curve. 12
Extraction and Positive Control Results and Interpretation 12
Summary 12
QUALITY CONTROL 13

INTENDED USE

The ProTect™ Omivar real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) kit is developed for qualitative detection of RNA from the SARS-CoV-2 Omicron variant. The ProTect™ Omivar RT-qPCR kit is validated to detect specifically for SARS-CoV-2 Omicron variant RNA in confirmed infected individuals or as a concurrent confirmatory test with ProTect™ COVID-19 RT-qPCR kit 2.0.

Positive results indicate the presence of SARS-CoV-2 Omicron variant RNA but do not rule out other infections (bacteria and other viruses) and the presence of SARS-CoV-2 Omicron variant RNA may not be the definite cause of disease. Negative results of the SARS-CoV-2 Omicron variant 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™ Omivar RT-qPCR kit is intended for use by trained laboratory personnel who has the proper skills to run RT-qPCR assays. The ProTect™ Omivar RT-qPCR kit is currently for research use only.

The ProTect™ Omivar RT-qPCR kit by JN Medsys provides all necessary reagents for the *in vitro* qualitative detection of SARS-CoV-2 Omicron variant from confirmed specimens and does not include reagents for the extraction and purification of RNA from the SARS-CoV-2 virus. The ProTect™ Omivar RT-qPCR kit is validated using Applied Biosystems® QuantStudio® 5 Real-Time PCR System and should also work on other Real-Time Systems with similar specifications.

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.

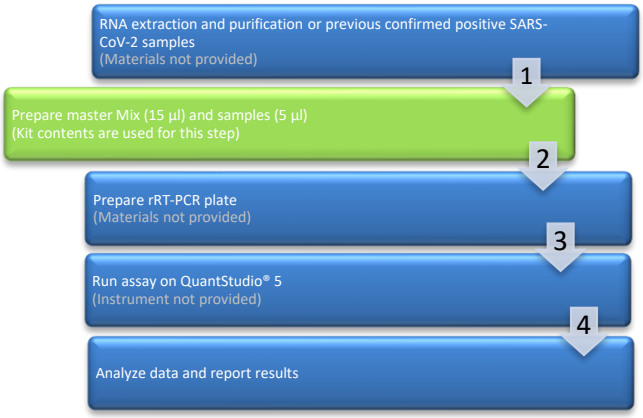
Since November 2021, the Omicron variant emerged as a variant of concern. The ProTect™ Omivar RT-qPCR kit is a molecular *in vitro* test that detects specifically for SARS-CoV-2 Omicron viral RNA. The detection of the viral RNA will aid in the identification of SARS-CoV-2 Omicron variant and is based 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 Omicron RNA in upper respiratory specimens.

PRINCIPLES OF THE PROCEDURE

The oligonucleotide primers and probes for detection of SARS-CoV-2 Omicron were selected from regions of the virus which have specific mutations to the Omicron variant. The kit is designed for the specific detection of 2 specific regions on the SARS-CoV-2 Omicron variant (two primer/probe sets).

The viral RNA is first extracted and purified from upper respiratory 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® 5 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



RECOMMENDED EQUIPMENT

Items	Details
qPCR instrument	Applied Biosystems® QuantStudio® 5 Real-Time PCR System Refer to manufacturer's instructions: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017162_QS5HIDInstrument_UG.pdf

WARNINGS AND PRECAUTIONS

- Patient specimens and positive controls should be 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.

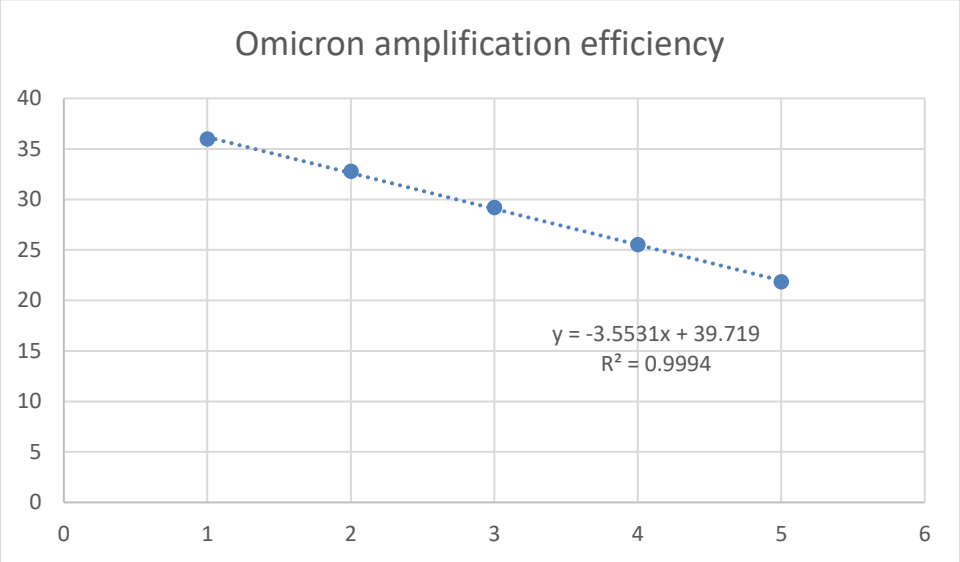
- Always check the expiration date and do not use expired reagents.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Use and change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory 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.

KIT FEATURES

Test Principle	One-step RT-qPCR (TaqMan®-based detection)
Targets	Omicron specific targets (FAM)
Number of Tests	100/kit
Compatible Specimen Type	Upper respiratory nasopharyngeal specimens
Limit of Detection	7.5 copies RNA per reaction
Amplification efficiency	>90%
Precision	<2%
Specificity	Detects only Omicron variant based on *in silico* sequence and wet lab validation

PERFORMANCE CHARACTERISTICS

Amplification efficiency:



Target	Amplification Efficiency (%)
Omicron	0.91

Limit of Detection (LoD):

Preliminary LoD studies determine the lower detectable concentration of SARS-CoV-2. The preliminary LoD was determined to be 7.5 copies/reaction based on limiting dilution studies. The results are summarised as follow:

Target	Concentration (copies/ reaction)	Number of replicates tested positive	Mean Ct	Standard deviation	RU (%)
Omicron	30	3/3	35.88	0.17	0.48
	15	3/3	36.89	0.27	0.74
	7.5	3/3	37.45	0.48	1.28

** Relative uncertainty = Standard deviation/Mean*

LoD studies determine the lowest detectable concentration of Omicron SARS-CoV-2 at which approximately 95% of all replicates test positive. The LoD was determined to be 7.5 copies/ reaction based on limiting dilution studies. 20 replicates of 15 copies / reaction, 7.5 copies / reaction and 3.75 copies / reaction were done, and the results are summarized as follow:

Target	Concentration (copies/ reaction)	Number of replicates tested positive	Mean Ct	Standard deviation	RU (%)
Omicron	15	20/20	35.03	0.47	1.34
	7.5	20/20	36.06	0.69	1.92
	3.75	17/20	37.39	0.86	2.31

** Relative uncertainty = Standard deviation/Mean*

Specificity

Omicron specific sequences were tested with wild type sequences to check if the primers and probes were specific. The Omicron specific sequences do not recognise wild type SARS-CoV-2. The results are summarised as follow:

Target	Twist Wildtype (dilution factor)	Mean Ct
Wild type SARS-CoV-2	10x	Undetermined
	100x	Undetermined
	1000x	Undetermined
	10000x	Undetermined
	100000x	Undetermined
	NTC	Undetermined

IN SILICO ANALYSIS OF PRIMER AND PROBE SEQUENCES

An alignment was performed with the oligonucleotide primer and probe sequences of the ProTect™ Omivar RT-qPCR kit with all publicly available complete nucleic acid sequences for SARS-CoV-2 Omicron variant in www.GISAID.org as from 22nd-28th Dec 2022 to demonstrate that the ProTect™ Omivar RT-qPCR kit was able to detect >99% of all known Omicron SARS-CoV-2 variants. All the alignments show that ProTect™ Omivar RT-qPCR kit was able to detect >99% of the available Omicron SARS-CoV-2 sequences with set criteria of detection of Omicron targets.

KIT CONTENTS

Each kit includes reagents sufficient to perform 100 tests (Cat No. 10035). Each test includes 1 duplex RT-qPCR assays, which target the Omicron on FAM.

Reagents Supplied	Tube Cap	100 Tests (10035) Volume (µL)
ProTect™ Probe qPCR Mastermix (2X)	Red-labelled	1 x 1000
ProTect™ RT Mix (50X)	Green-labelled	1 x 45
ProTect™ Omivar Primer & Probe Mix	Purple-labelled	1 x 510
ProTect™ Omivar Positive Control^*	Yellow-labelled	1 x 110
Nuclease Free Water	Blue cap	1 x 2000

^Sufficient for 20 runs
** The ProTect™ Omivar Positive Control is made up of synthetic Omicron sequences. This serves as a control for the Omicron test.*

STORAGE AND STABILITY

The ProTect™ Omivar RT-qPCR kit 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.

ASSAY SETUP

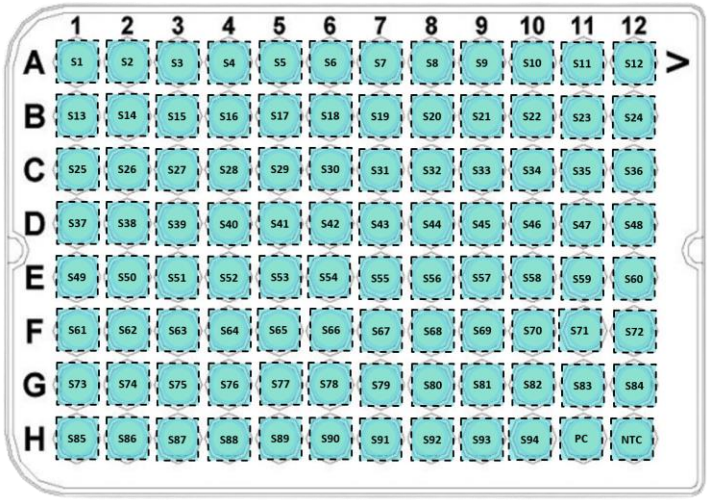
1. Thaw reagents at room temperature and maintain reagents on ice when thawed. Mix reagents gently and briefly centrifuge to collect contents at the bottom of the tubes.
2. Prepare each reaction mix as shown in the table below:

No.	Reagents	Volume (µL)
1	ProTect™ Probe qPCR Mastermix (2X) [Red-labelled]	10
2	ProTect™ RT Mix (50X) [Green-labelled]	0.4
3	ProTect™ Omivar Primer & Probe Mix [Purple-labelled]	4.6
4	RNA Sample/ ProTect™ Omivar 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

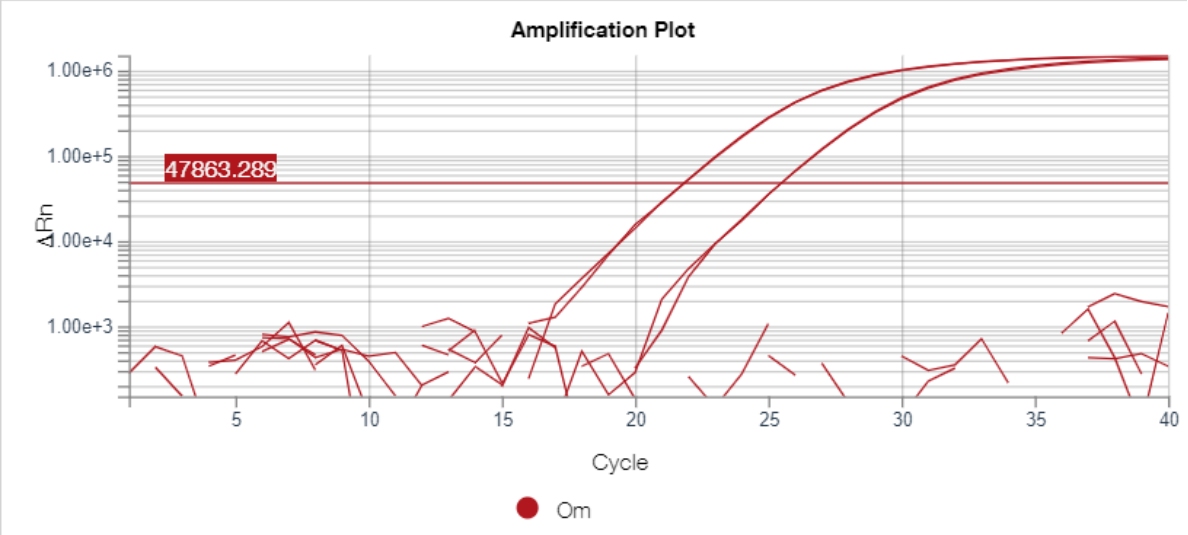
4. Centrifuge to collect contents at the bottom of the tube strip/plate.
5. Transfer tube strip/plate to qPCR instrument
6. For QuantStudio® 5 Real-Time PCR System, refer to user manual for machine operation and experimental setup (https://assets.thermofisher.com/TFS-

Assets/LSG/manuals/MAN0017162_QS5HIDInstrument_UG.pdf). *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 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

https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-detection-instructions.html



DATA ANALYSIS AND INTERPRETATION

Interpretation of ProTect™ Omivar RT-qPCR kit 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 Omicron Positive Control (PC)

   The PC consists of synthetic Omicron target sequences. The PC will yield a positive result with the following primer and probe sets: ProTect™ Omivar Primer & Probe Mix.

3. SARS-CoV-2 Omicron Marker (Om)

   When all controls exhibit the expected performance, a specimen is considered positive if Om cycle threshold is less than 40 cycles (< 40 ct).

   When all controls exhibit the expected performance, a specimen is negative for SARS-CoV-2 Omicron variant when SARS-CoV-2 Omicron target Om cycle threshold are more than 40.00 cycles (> 40) and the cycle threshold.

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:

Om (FAM)	Outcome
+	SARS-CoV-2 Omicron detected
-	SARS-CoV-2 Omicron not detected

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.

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.

• The ProTect™ Omivar RT-qPCR kit is intended only for use with upper respiratory specimens (nasopharyngeal 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 Omicron variant.

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

Revision History

Revision	Effective Date	Description of Change
01	11 Jan 2022	1. Initial release for use
02	07 Jul 2022	2. Uprevision of the footer and added document number

