TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit

COVID-19 Real-Time Multiplex Direct RT-PCR Test

Instructions for Use

IVD

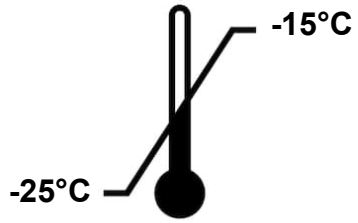
REF SKU#TP-002-02

Σ 100

Important!

The instructions for use must be read carefully prior to use and followed strictly to achieve reliable results. Any deviations from the protocol will have a significant impact

Storage and Transport Conditions



(Protect from light during storage and transport)

cellbae

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Contents

1 Intended Use

The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit is a direct real-time reverse transcriptase polymerase chain reaction (RT-PCR) technology for the qualitative and simultaneous detection of 2 genetic targets of SARS-CoV-2-specific RNA and 1 genetic target of human-specific mRNA from Universal Transport Media. A positive result from the test may indicate the presence of SARS-CoV-2-specific RNA in the test sample. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of SARS-CoV-2 infection.

You should not rely on or otherwise use the results as the sole means for clinical diagnosis and treatment. By using TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit, you agree to the terms and conditions set out in this document.

2 Kit Components

Component Label	Component	Storage Temp	Number of Vials	Volume [µL/Vial]
1 (TP-002-02-TU1)	Master Mix A	– 20ºC	1	1250
2 (TP-002-02-TU2)	Master Mix B	– 20ºC	1	150
3 (TP-002-02-TU3)	Internal & Positive Controls Mix	– 20ºC	1	50
4 (TP-002-02- TU4)	Lysis Reagent	– 20ºC	1	500
5 (TP-002-02-TU5)	Water	– 20ºC	1	1250

To avoid contamination of positive and internal control templates, users are advised to make working aliquot of each component.

3 Storage

- The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit is shipped in dry ice. The components of the kit should arrive frozen. If one or more components is not frozen upon receipt, or if vials have been compromised during shipment, contact Technical Support (refer to Section 13) for assistance.
- All components should be stored between –25°C and –15°C upon arrival.
- Protect from light.

4 Material and Devices required but not provided

Note: *The names of vendors or manufacturers are provided as examples of suitable product sources. Users have to conduct verification to determine whether to use the product sources provided herein or other product sources.*

- Appropriate oropharyngeal (OP) and/or nasopharyngeal (NP) and/or midturbinate (MT) swabs for biological specimen collection in Universal Transport Medium (UTM). A negative result from the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit may be due to failed specimen collection. Interpretation of test result should take into consideration of available clinical information.
- The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit has been used on UTM samples from human NP and/or OP and/or MT swabs.
- Appropriate RT-PCR machine (see Section 5.1).
- Benchtop centrifuge with a rotor for 1.5- and 2-mL reaction tubes.
- Centrifuge that goes up to 1,000 rcf with a rotor for 96-well microtiter plates or 8-well strips.
- Appropriate 96-well reaction plates or reaction tubes with corresponding (optical) closing material.
- Pipettes (adjustable).
- Pipette tips with filters (disposable).
- Powder-free gloves (disposable).

- Vortex mixer.
- Cellbae Direct RT-PCR System for Automatic Setup (optional)

NOTE	
i	***Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***

5 Product Description

- The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit is an *in vitro* diagnostic (IVD) test kit. This assay has received Provisional Authorisation from the Health Sciences Authority in Singapore.
- The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit is based on real-time RT-PCR technology, for the qualitative and simultaneous detection of 2 genetic targets of SARS-CoV-2-specific RNA and 1 genetic target of human-specific mRNA. The assay includes a no template control (PCR Grade Water), a positive control and an endogenous internal control that functions as an extraction control for human clinical samples.
- Real-time RT-PCR technology utilizes (1) reverse transcription (RT) reaction to convert RNA into complementary DNA (cDNA), (2) PCR to amplify specific genetic target sequences, and (3) target-specific probes to detect amplified DNA. The probes are labelled with fluorescent dyes and quenchers.
- Probes specific for SARS-CoV-2 RNA are labelled with the fluorophores 6-FAM or Cy5. The probe specific for human Internal Control (IC) is labelled with the fluorophore HEX.
- The test consists of four processes in a single assay:
    - Removal of inhibitors in UTM for Direct RT-PCR, OR RNA extraction for RT-PCR with RNA isolation
    - Reverse transcription of target RNA to cDNA
    - PCR amplification of target and Internal Control templates
    - Simultaneous detection of PCR amplicons by probes that are

labelled with fluorescent dyes and quenchers

5.1 Real-Time PCR Instrument

The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit was developed and validated to be used with the following real-time PCR instruments:

- BIO-RAD CFX96™ Real-Time PCR Detection System

The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit may not deliver the same results if used with other systems.

5.2 Automatic Direct RT-PCR Setup System

The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit was developed and validated to be used with the following automatic Direct RT-PCR Setup Instrument:

- Cellbae™ Direct RT-PCR Setup System

For additional information and technical support regarding the use of the Automatic Direct RT-PCR Setup System, please contact our Technical Support (see Section 13).

6 Warnings and Precautions

Read the Instructions for Use carefully before using the product.

a. Before first use, please check the product and its components for:
   i. Integrity
   ii. Completeness with respect to number, type and filling (see Section 2 Kit Components)
   iii. Correct labelling
   iv. Frozenness upon arrival

b. Use of this product is limited to personnel specially instructed and

trained in the techniques of real-time RT-PCR.

c. Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
d. Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
e. Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
f. Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
g. Always wear protective disposable powder-free gloves when handling kit components.
h. Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
i. Dedicate supplies and equipment for the individual working areas and do not move them from one area to another.
j. Store positive and/or potentially positive material separated from all other components of the kit.
k. Do not open the reaction tubes/plates post amplification to avoid amplicon contamination.
l. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
m. Do not autoclave reaction tubes after the PCR. This will not degrade the amplified nucleic acid but risk contaminating the laboratory area.
n. Do not use components of the kit that have passed their expiration date.
o. Discard sample and assay waste according to your local safety regulations.

7 Procedure

7.1 Preparation of Swab samples

- Fresh UTM with an OP and/or NP and/or MT patient swab is the starting material for the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit.
- The TEPAT 2.0 SARS-CoV-2 Multiplex RT-PCR Kit has been validated using full length SARS-CoV-2 viral RNA spiked into Kang Jian Collection Swab & Virus Sampling tube with fresh patient OP and MT swabs (COPAN FLOQSwabs and Kang Jian Collection Swab) as well as RNA eluate of NP swabs.
- Compatibility of other nucleic acid extraction system/procedure with TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit has to be validated by the user.
- The UTM has a significant impact on the performance of downstream RT-PCR. User must ensure that the UTM used for sample collection is compatible with Direct RT-PCR technology.
- The HEX channel of the assay functions as an **extraction control** to assess RNA extraction efficiency from inhibitor removal from UTM samples for Direct RT-PCR in human clinical samples only.
- A negative result from the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit may be due to failed sample preparation. Interpretation of assay result should take into consideration available clinical information.

7.2 RNA Extraction or Inhibitor Removal from UTM

RNA may first be extracted from the swab samples using a commercial kit following respective manufacturer's instructions. The TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit has been validated on samples extracted with the Liferiver EX3600 extraction system. Section 7.2.1 details instructions for processing of UTM for direct RT-PCR.

7.2.1 Inhibitor Removal from UTM for Direct RT-PCR (Option)

- Bring the Lysis Reagent (TP-002-02-TU4) to room temperature, and centrifuge before use

- Add 5 µL of Lysis Reagent in a 0.2-mL PCR tube
- Add 70 µL of UTM sample into 5 µL of Lysis Reagent
- Vortex to mix and leave at room temperature for at least 10 min
- For additional information and technical support regarding pre-treatment and sample preparation, please contact our Technical Support (see Section 13)

7.3 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use. The TEPAT 2.0 SARS-CoV-2 Multiplex RT-PCR Kit contains an endogenous Internal Control (IC), which serves as a control of the sample preparation procedure and as an RT-PCR inhibition control. Do not combine components of the test kit with those from different lot numbers.

The Master Mix can be set up as follows:

7.3.1 For Direct RT-PCR (Following Sample Preparation as per Section 7.2.1):

Number of Reactions	1	10	100
Master Mix A	12.5 µL	125 µL	1250 µL
Master Mix B	1.5 µL	15 µL	150 µL
Water	9.75 µL	97.5 µL	975 µL
Volume of Master Mix	23.75 µL	237.5 µL	2375 µL

7.3.2 For RT-PCR with RNA Eluate:

Number of Reactions	1	10	100
Master Mix A	12.5 µL	125 µL	1250 µL
Master Mix B	1.5 µL	15 µL	150 µL
Volume of Master Mix	14 µL	140 µL	1400 µL

7.4 Reaction Setup

Ensure that at least one Positive Control and one Negative Control are used per run. Thoroughly mix the samples and controls with the Master Mix by pipetting up and

down. Close the 96-well reaction plate with appropriate lids or optical adhesive film. Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1,000 rcf (~ 3,000 rpm).

7.4.1 For Direct RT-PCR:

Reaction Setup			
	Sample Assay	Positive Control	No Template Control (NTC) / Negative Control
Master Mix	23.75 µL	23.75 µL	23.75 µL
Sample	1.25 µL	0	0
Clean UTM (NTC) or True Negative UTM sample	0	0	1.25 µL
Internal and Positive Controls Mix	0	1.25 µL	0 µL
Total Volume	25 µL	25 µL	25 µL

7.4.1.1 Addition of Master Mix

Pipette 23.75 µL of the Master Mix into each required well of an appropriate optical 96-well reaction plate (recommended: Bio-Rad Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white for use on BIO-RAD CFX96™ Real-Time PCR Detection System); or an appropriate optical reaction tube.

7.4.1.2 Addition of samples

Add 1.25 µL of the sample (UTM treated for inhibitor removal) and complete the reaction volume to 25 µL.

7.4.1.3 Addition of controls

Add 1.25 µL of the Internal and Positive Controls Mix provided with the TEPAT 2.0 SARS-CoV-2 Multiplex RT-PCR Kit into the wells that will be used for the Positive Control. Add 1.25 µL of clean UTM used in sample collection or a true negative UTM sample (not provided) as a negative control.

7.4.2 For RT-PCR with RNA Eluate:

Reaction Setup			
	Sample Assay	Positive Control	No Template Control (NTC) / Negative Control
Master Mix	14 µL	14 µL	14 µL
Sample RNA eluate	11 µL	0	0
Water (NTC) or True Negative RNA eluate	0	8.5 µL	11 µL
Internal and Positive Controls Mix	0	2.5 µL	0 µL
Total Volume	25 µL	25 µL	25 µL

7.4.2.1 Addition of Master Mix

Pipette 14 µL of the Master Mix into each required well of an appropriate optical 96-well reaction plate (recommended: Bio-Rad Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white for use on BIO-RAD CFX96™ Real-Time PCR Detection System; Roche Diagnostics LIGHTCYCLER 480 PLATE 96 WHITE for use on LightCycler® 480 Instrument II Real-Time PCR System); or an appropriate optical reaction tube.

7.4.2.2 Addition of samples and controls

Add 2.5 µL of the Internal and Positive Controls Mix provided with the TEPAT 2.0 SARS-CoV-2 Multiplex RT-PCR Kit into the wells that will be used for the Positive Control.

Add 2.5 µL of water or an RNA eluate from a true negative clinical specimen (not provided) as a negative control and complete the reaction volume to 25 µL with water.

Add 11 µL of the sample (RNA eluate) and complete the reaction volume to 25 µL with water.

8 Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit on specific real-time PCR instruments please contact our Technical Support (see Section 13).

8.1 Temperature Profile and Dye Acquisition

Step	Description	Temperature	Duration (Min:Sec)	No. of Cycles	Detection
1	Reverse Transcription (RT)	42°C	04:00	1	-
2	RT Inactivation / Initial Denaturation	95°C	00:30	1	-
3	Denaturation	98°C	00:05	45	-
4	Annealing [Data Collection]	59.0°C	01:20		FAM, HEX and Cy5

8.2 Fluorescence Dyes

Target name	Reporter	Quencher
SARS-CoV-2 I	6-FAM	BHQ-1
Internal Control	HEX	BHQ-1
SARS-CoV-2 II	Cy5	BHQ-2

8.3 Special Remarks on the Setup of the CFX96™ Systems

Define the following settings:

Sample volume	25 µL
Plate type	Choose white/clear appropriately according to optical reaction tube/plate used

Target	Detector Name	Reporter
SARS-CoV-2 RNA I	RdRP gene	6-FAM
Internal Control	IC	HEX
SARS-CoV-2 RNA II	NSP8 gene	Cy5

Open the “Plate Setup” window and select “View / Edit Plate...”. Select all wells of the 96-well plate. Click “Select Fluorophores”. For “Channel 1” check the box behind FAM, for “Channel 2” check the box behind HEX, and for “Channel 4” check the box behind Cy5. Assign samples to the wells by selecting the appropriate “Sample Type” and afterwards “Load” FAM and HEX to the wells. The target name of FAM should be set to “RDRP”, the target name of HEX should be set to “IC” and the target name of Cy5 should be set to “NSP8”.

9 Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For questions regarding data analysis of TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit on authorized real-time PCR instruments please contact our Technical Support (see section 13).

9.1 Validity of Diagnostic Test Runs

9.1.1 Valid Test Run

For a valid diagnostic test run, the following control conditions must be met:

Control ID	FAM Detection Channel (RdRP)	HEX Detection Channel (Internal Control)	Cy5 Detection Channel (NSP8)
Positive Control	C_t or C_p <40	C_t or C_p <40	C_t or C_p <40
No Template Control (clean UTM or water only)	No C_t or C_p	No C_t or C_p	No C_t or C_p
Negative Process Control (UTM or RNA eluate of a true negative sample)	No C_t or C_p	C_t or C_p <40	No C_t or C_p

9.1.2 Invalid Test Run

A diagnostic test run is invalid, (i) if the run has not been completed or (ii) if any of the control conditions for a valid diagnostic test run are not met. In the case of an invalid diagnostic test run, repeat testing by using the remaining UTM (with OP and/or NP and/or MT patient swab), or purified nucleic acids, or start from the original samples again. If a test run is repeatedly invalid, please contact our Technical Support (see section 13).

9.2 Interpretation of Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If one or more controls are not valid, the patient results cannot be interpreted.

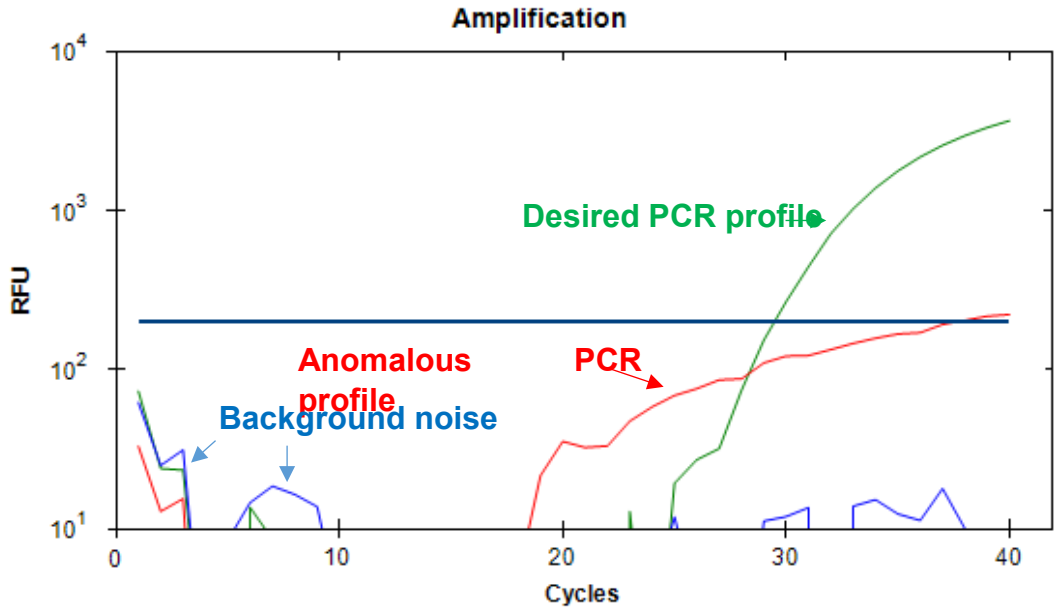
FAM (RdRP gene)	HEX (Internal Control)	Cy5 (NSP8 gene)	Result Interpretation
C_t or C_p <40	Any or no C_t or C_p*	Any or no C_t or C_p	SARS-CoV-2 specific RNA detected. Positive for SARS-CoV-2. Report result to healthcare provider and appropriate public health authorities
Any or no C_t or C_p	Any or no C_t or C_p*	C_t or C_p <40	
No C_t or C_p	C_t or C_p < 40	No C_t or C_p	No SARS-CoV-2 specific RNA detected. The sample does not contain detectable amounts of SARS-CoV-2 specific RNA. Report result to healthcare provider.
No C_t or C_p	No C_t or C_p	No C_t or C_p	Swab failure or RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample

* Detection of the Internal Control in the HEX detection channel can be reduced (i.e. C_t or C_p > 40) or absent (i.e. no C_t or C_p) due to a high SARS-CoV-2 RNA load in the sample.

- As per standard RT-PCR data analysis, interpretation of TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit test result should take into consideration of the **C_t values**, as well as the shape of the **fluorescence growth curves** in the PCR amplification step as shown in the following figure:
- The C_t on a CFX96 system should be determined using a baseline threshold RFU cut-off at 100 for FAM, 25 for HEX and 25 for Cy5 with a Baseline Subtracted Curve Fit and Application of Fluorescence Drift Correction. Using

an automatic baseline threshold with a Baseline Subtracted Curve Fit and Application of Fluorescence Drift Correction is also accepted.

- Results of the assay should be interpreted in consideration of available clinical information.



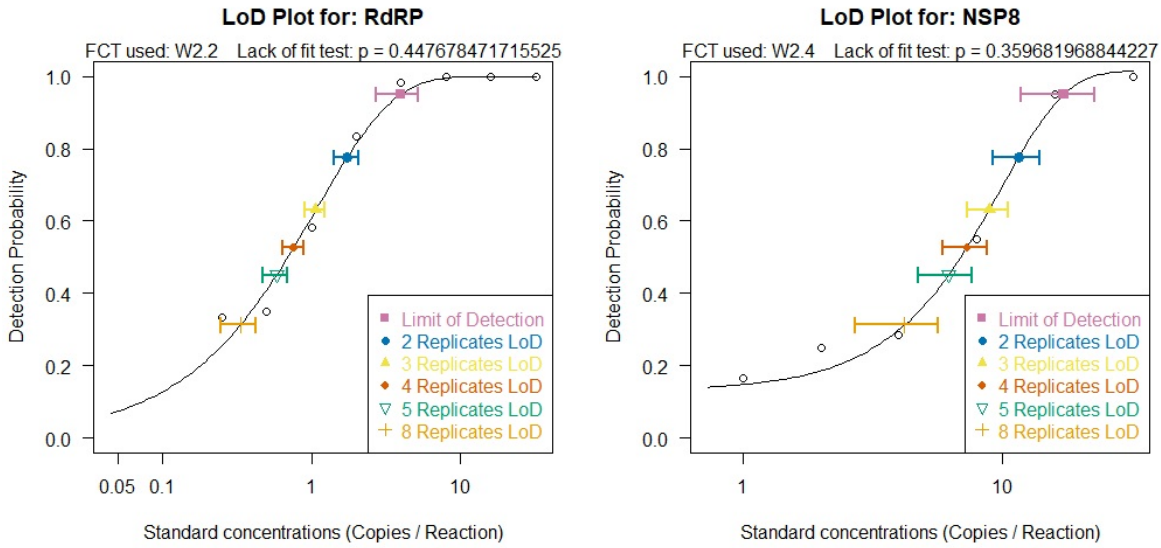
10 Performance Evaluation

Performance evaluation of the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit was done using quantified SARS-CoV-2 RNA spiked into UTM with OP and MT swabs from true negative clinical specimens for Direct RT-PCR and was done using quantified SARS-CoV-2 RNA spiked into RNA eluate of NP swab samples of true negative clinical specimens.

10.1 Analytical Sensitivity

10.1.1 Limit of Detection (LoD) with RNA Eluate

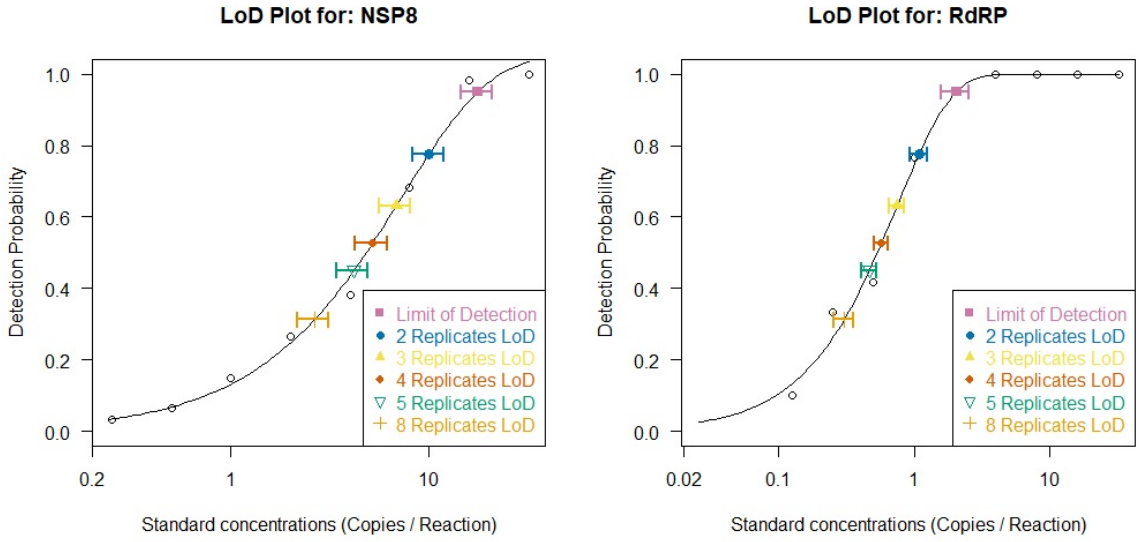
The experimental design is used to process replicates of dilutions made from three independent samples of known measurand concentration across 3 days. 30 unique negative patient specimens were obtained and run in duplicates across all testing days.



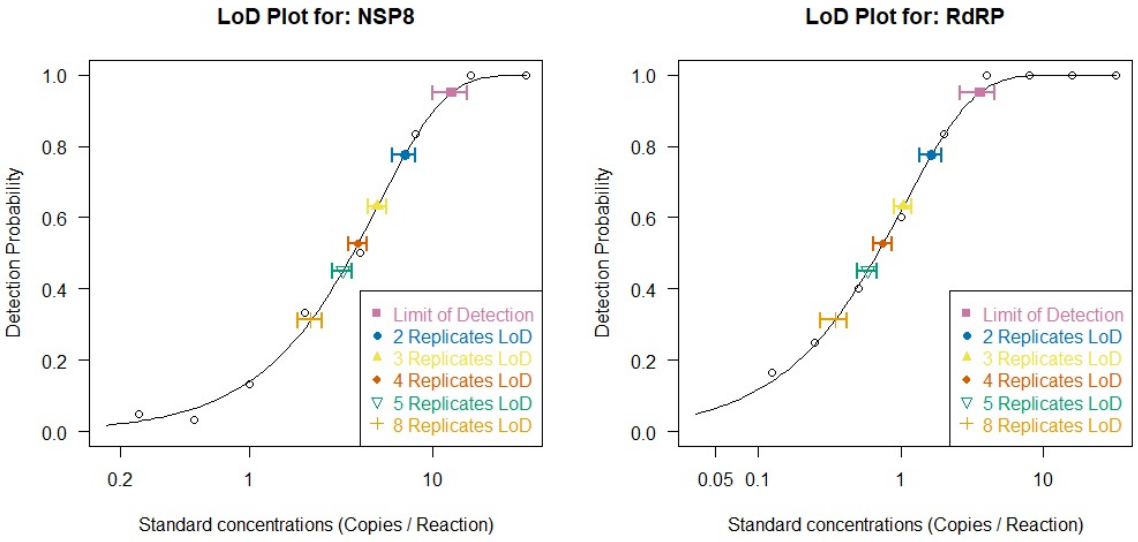
An estimate of 4.0 copies/reaction is reported for the measurement procedure for the RdRP target and 17.4 copies/reaction is reported for the measurement procedure for the NSP8 target.

10.1.2 Limit of Detection (LoD) Direct RT-PCR (Manual)

The experimental design is used to process replicates of dilutions made from three independent samples of known measurand concentration.



10.1.3 Limit of Detection (LoD) Direct RT-PCR (Automated)



An estimate of 2.0 copies/reaction is reported for the measurement procedure for the RdRP target and 17.7 copies/reaction is reported for the measurement procedure for the NSP8 target when Direct RT-PCR was setup manually.

An estimate of 3.6 copies/reaction is reported for the measurement procedure for the RdRP target and 12.7 copies/reaction is reported for the measurement procedure for the NSP8 target when Direct RT-PCR was setup automatically using the Cellbae Direct RT-PCR System.

10.2 Inclusivity

10.2.1 In silico analysis on all SARS-CoV-2 sequences published on GISAID database

Table 2. Inclusivity – In silico analysis of 1,388,138 whole genome sequences of SARS-CoV-2 published in GISAID e.V. (www.gisaid.org) as of 15 May 2021 for the RdRP and NSP8 gene targets: TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit.

Whole Genome Sequences		Identity	Comment
RdRP gene	Forward Primer, Reverse Primer and Probe	99.3%	1,378,322 sequences: 100% identity 9,816 sequences: 1 base mismatch or more
NSP8 gene		99.6%	1,382,230 sequences: 100% identity 5,908 sequences: 1 base mismatch or more
RdRP +		100%	1,388,138 sequences: 100% identity with either

NSP8 gene duplex			RdRP or NSP8 target regions

Genomes on the GISAID database included sequences from the NCBI database. From the analysis, a single oligonucleotide sequence mutation event leading to ≤ 2 mismatches will not have any significant negative impact on the amplification of the respective target sequences.

A total of 9,816 sequences do not share 100% identity with the RdRP gene assay and 5,908 sequences do not share 100% identity with the NSP8 gene assay. However, taken together reactivity of the specific oligonucleotide included in the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit towards SARS-CoV-2 RNA detection is not expected to be affected.

10.2.2 Statement on coverage of emerging SARS-CoV-2 Variants

The new SARS-CoV-2 viral mutations: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1) and Delta (B.1.617) variants are available on the GISAID database as of 15 May 2021. We have found that these variants remain detectable by our assay as per our in silico analysis.

10.2.3 Limit of Blank (LoB)

Negative patient samples and water and UTM were used to confirm that LoB is zero.

10.3 Cross-reactivity (*in silico*)

The Sequences of the primers and probes in the TEPAT 2.0 SARS-CoV-2 Multiplex RT-PCR Kit were blasted against the following species:

1. SARS-coronavirus
2. MERS-coronavirus
3. Influenza A
4. Adenoviruses

5. *Chlamydia pneumoniae*
6. Human coronavirus 229E
7. *Haemophilus influenzae*
8. Human coronavirus OC43
9. *Legionella pneumophila*
10. Human coronavirus HKU1
11. *Mycobacterium tuberculosis*
12. Human coronavirus NL63
13. *Streptococcus pneumoniae*
14. MERS-coronavirus
15. *Streptococcus pyogenes*
16. Adenovirus
17. *Bordetella pertussis*
18. Human metapneumovirus (hMPV)
19. *Mycoplasma pneumoniae*
20. Parainfluenza virus 1-4
21. *Pneumocystis jirovecii* (PJP)
22. Influenza A virus
23. *Candida albicans*
24. Influenza B virus
25. *Pseudomonas aeruginosa*
26. Enterovirus
27. *Staphylococcus epidermidis*
28. Respiratory syncytial virus
29. *Streptococcus salivarius*
30. Rhinovirus

The search parameters used for the BLAST analysis were set to the following: max target sequences: 20000, short queries, automatically adjust parameters for short input sequences, expect threshold: 10, word size: 16, max matches in a query range: 0, match/mismatch scores: 2,-3, low complexity regions filter.

Hits were reviewed for potential formation of PCR product through binding of the primers in close proximity and with the right orientation to each other on target

nucleic acid molecules. No combination that could lead to undesired amplification of potentially cross-reacting target sequences was found.

10.4 Precision

10.4.1 Repeatability/Reproducibility

Low-level samples of 3 sample lot dilutions were run over 4 days amounting to at least 20 replicates per low-level sample type. Samples were produced by spiking known measurand of RNA standard into negative RNA eluates.

NSP8 Copies/Rxn	Mean Ct	SD	% CV	% Replicate Detection
32	31.8	0.544	1.71	100
16	32.9	0.748	2.28	93
8	34.5	1.638	4.75	55
4	34.9	1.028	2.95	27
2	35.7	1.533	4.30	20
1	36.5	0.804	2.20	10
0.5	34.8	3.660	10.52	5
0.25	N/A	N/A	N/A	0

RdRP Copies/Rxn	Mean Ct	SD	% CV	% Replicate Detection
32	31.7	0.705	2.22	100
16	32.8	0.808	2.46	100
8	34.1	1.053	3.09	100
4	35.8	1.721	4.81	100
2	37.0	1.702	4.60	73
1	37.3	1.406	3.77	42
0.5	37.7	0.939	2.49	18
0.25	38.0	1.288	3.39	15

Low-level samples of 3 sample lot dilutions were run over 3 days. Samples were produced by spiking known measurand of RNA standard into fresh pooled negative samples and processed manually using the lysis protocol (Section 7.2.1).

NSP8 **Copies/Rxn**	**Positives Detected**	**# Tests**	**Mean Ct**	**SD**	**% CV**	**% Replicate Detection**
32	60	60	32.4	0.615	1.90	100.0
16	59	60	33.7	0.754	2.24	98.3
8	41	60	34.7	0.987	2.84	68.3
4	23	60	35.5	0.878	2.47	38.3
2	16	60	36.0	0.552	1.53	26. 7

1	8	60	37.4	2.238	5.99	13.3
0.5	4	60	36.8	0.109	0.30	6.7
0.25	2	50	36.5	0.134	0.37	4.0
0.125	0	60	N/A	N/A	N/A	0.0
0	0	60	N/A	N/A	N/A	0.0

RdRP **Copies/Rxn**	**Positives Detected**	**# Tests**	**Mean Ct**	**SD**	**% CV**	**% Replicate Detection**
32	60	60	31.2	0.266	0.85	100.0
16	60	60	32.4	0.340	1.05	100.0
8	60	60	33.7	0.587	1.74	100.0
4	60	60	34.8	0.724	2.08	100.0
2	57	60	36.0	1.021	2.83	95.0
1	46	60	36.9	1.232	3.34	76.7
0.5	25	60	37.7	1.075	2.85	41.7
0.25	17	60	37.9	1.103	2.91	28.3
0.125	6	60	38.2	0.970	2.54	10.0
0	0	60	N/A	N/A	N/A	0.0

Low-level samples of 3 sample lot dilutions were run over 4 days. Samples were produced by spiking known measurand of RNA standard into fresh pooled negative samples and processed automatically using the Cellbae Direct RT-PCR System (Section 7.2.1).

NSP8 **Copies/Rxn**	**Positives Detected**	**# Tests**	**Mean Ct**	**SD**	**% CV**	**% Replicate Detection**
32	60	60	33.0	0.867	2.63	100.0
16	60	60	34.4	1.067	3.10	100.0
8	51	60	35.4	1.113	3.15	85.0
4	30	60	36.0	1.167	3.24	50.0
2	20	60	36.5	1.152	3.15	33.3
1	8	60	36.5	0.465	1.27	13.3
0.5	2	60	37.6	1.527	4.06	3.3
0.25	3	50	37.3	0.174	0.47	6.0
0.125	1	60	36.8	N/A	N/A	1.7
0	0	60	N/A	N/A	N/A	0.0

RdRP **Copies/Rxn**	**Positives Detected**	**# Tests**	**Mean Ct**	**SD**	**% CV**	**% Replicate Detection**
32	60	60	31.7	0.654	2.06	100.0
16	60	60	33.1	0.745	2.25	100.0
8	60	60	34.5	1.023	2.96	100.0
4	60	60	35.8	1.189	3.32	100.0

2	50	60	36.8	1.083	2.94	83.3
1	36	60	37.3	1.132	3.04	60.0
0.5	24	60	38.0	0.758	2.00	40.0
0.25	15	60	38.1	0.612	1.60	25.0
0.125	10	60	38.3	0.718	1.88	16.7
0	0	60	N/A	N/A	N/A	0.0

10.5 Potential Interfering Substance

Potential interfering substances from respiratory specimens were tested for ability to generate false negative results using samples containing the extracted viral RNA at 3x LoD in nuclease free water. In addition, potential interfering substances from respiratory specimens were introduced to negative samples and tested for the ability to generate false positive results. Testing was performed with 3 replicates per substance for both RT-PCR from RNA eluate and for direct RT-PCR.

Potential Interfering Substances	Active Ingredient	Tested Concentration	Positive Samples		Negative Samples	
			COVID-19 % Detection (# Detected / # Tested)	HEX % Detection	COVID-19 % Detection (# Detected / # Tested)	HEX % Detection
Bovine submaxillary gland mucin, type 1-5	Mucin	1.25 mg/mL	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Blood	Blood	10% (v/v)	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Anti-viral drug	Zanamivir	7.5 mg/mL	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Anti-viral drug	Oseltamavir	15 mg/mL	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Systemic antibiotic	Tobramycin	50 µg/mL	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Antibiotic nasal ointment	Mupirocin	1.65 mg/mL	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Nasal spray	Oxymetazoline	25% (v/v)	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Nasal gel	Sodium hyaluronate	12.5% (v/v)	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)
Throat lozenges	2,4-Dichlorobenzyl	25% (v/v)	100% (3/3)	100% (3/3)	0% (3/3)	100% (3/3)

	alcohol, Amylmetacresol					

11 Limitations

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurities and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the UTM specimen directly. Inhibitor removal step (refer to step 7.1 above) must be conducted prior to using this assay in Direct RT-PCR mode.
- The presence of RT-PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- A false negative result may occur if inadequate numbers of the target organism (SARS-CoV-2) are present in the specimen due to improper collection, transport, or handling.
- As with any test, results of the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.
- RNA viruses in particular show substantial genetic variability. Although continuous efforts were made to monitor potential mutation in the target regions that might result in mismatches between the primers, probes, and the target sequences based on available viral sequences information, onset of new mutation can result in diminished assay performance and possible false negative results.

12 Quality Control

- To ensure consistent product quality, each lot of the TEPAT 2.0 SARS-CoV-2 Multiplex Direct RT-PCR Kit is tested against predetermined specifications.
- Users are strongly discouraged to combine components from assay kits of different lot numbers.

13 Technical Assistance

For technical advice, please contact our Technical Support:
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Tel: +65 6824 7036

14 Trademarks and Disclaimers

Product regulatory cleared or approved. Not available in all countries.

15 Explanation of Symbols

IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade identification number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version