TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit

COVID-19 Real-Time RT-PCR Test

Instructions for Use

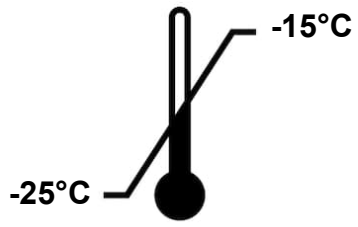
IVD

REF SKU#TP-001-01

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)

Kit has shelf-life of 6 months if stored within this temperature range.

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Contents

1 Intended Use

The TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit is a 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 nasopharyngeal swabs. 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.

This assay has received Provisional Authorisation from the Health Sciences Authority in Singapore.

You should not rely on or otherwise use the results as the sole means for clinical diagnosis and treatment. By using TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit, you agree to the terms and conditions set out in this document.

2 Kit Components

Component Label	Lid Colour	Component	Storage Temp	Number of Vials	Volume [µL/Vial]
1 (TP001-01-TU1)	Amber	Master Mix A	–20ºC	1	1250
2 (TP001-01-TU2)	Blue	Master Mix B	–20ºC	1	150
3 (TP001-01-TU3)	Red	Positive Control	–20ºC	1	100
4 (TP001-01-TU4)	Yellow	Internal control	–20ºC	1	100

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

3 Storage

- The TEPAT 1.0 SARS-CoV-2 Multiplex 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 or other product sources.*

- Appropriate nasopharyngeal swabs for biological specimen collection. A negative result from the TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit may be due to failed specimen collection. Interpretation of test result should take into consideration available clinical information.
- Appropriate nucleic acid extraction kit (see Section 7.1).
- The TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit has been used on RNA samples extracted with Liferiver Bio-Tech EX3600 nucleic acid extraction systems.
- 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.

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 1.0 SARS-CoV-2 Multiplex RT-PCR Kit is an in vitro-diagnostic (IVD) test kit which has received Provisional Authorisation from the Health Sciences Authority in Singapore.
- The TEPAT 1.0 SARS-CoV-2 Multiplex 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 negative control (PCR Grade Water, not provided), a positive control and an endogenous internal control that functions as an extraction control for human clinical samples.
- Real-time RT-PCR technology utilizes reverse transcription (RT) reaction to convert RNA into complementary DNA (cDNA), PCR to amplify specific genetic target sequences, and 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 and Quasar 670. The probe specific for Internal Control (IC) is labelled with the fluorophore HEX.
- The test consists of three processes in a single assay:
    - 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 Instruments

The TEPAT 1.0 SARS-CoV-2 Multiplex 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
- LightCycler® 480 Instrument II Real-Time PCR System

The TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit may not deliver the same results if used with other systems.

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 RNA Sample Preparation

- Extracted RNA is the starting material for the TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit.
- The TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit has been validated using RNA samples extracted from clinical nasopharyngeal swabs via the Liferiver Bio-Tech EX3600 nucleic acid extraction system.
- Compatibility of other nucleic acid extraction system/procedure with TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit has to be validated by the user.
- The quality of the extracted RNA has a significant impact on the performance of downstream RT-PCR. User must ensure that the system used for nucleic acid extraction is compatible with RT-PCR technology.
- The HEX channel of the assay functions as an **extraction control** to assess RNA extraction efficiency for the RNA extraction protocol of choice in human clinical samples only.
- A negative result from the TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit

may be due to failed sample preparation. Interpretation of assay result should take into consideration available clinical information.

CAUTION	
⚠	***If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.*** ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

- For additional information and technical support regarding pre-treatment and sample preparation, please contact our Technical Support (see Section 13).

7.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use. The TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit contains an endogenous Internal Control (IC), which serves as a control of the sample preparation procedure (nucleic acid extraction) and as an RT-PCR inhibition control. Do not combine components of the test kit with those from a different lot numbers.

The Master Mix can be set up as follows:

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

7.3 Reaction Setup

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.

Add 5 µL of the Internal Control provided with the TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit into the wells that will be used for the Negative Control (Water, PCR grade, not provided) and the Positive Control (red-labelled cap). Do not add additional Internal Control template into the wells that will be used with any sample or control, which has been extracted previously. Users need to do their own nucleic acid extraction prior to RT-PCR and are recommended to use the Liferiver MVR 01 kit.

Add 10 µL of the sample (eluate from the nucleic acid extraction) or 5 µL of the controls (Positive or Negative Control) and complete the reaction volume to 25 µL with Water (PCR grade).

Reaction Setup			
	Sample Assay	Positive Control	Negative Control
Master Mix	14 µL	14 µL	14 µL
Sample	10 µL	0	0
Water	1 µL	1 µL	6 µL
Internal Control	0	5 µL	5 µL
Positive Control	0	5 µL	0
Total Volume	25 µL	25 µL	25 µL

Ensure that at least one Positive Control and one Negative Control is 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).

Do not combine components of the test kit with those from a different lot number in the same reaction setup.

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 1.0 SARS-CoV-2 Multiplex 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	05:00	1	-
2	RT Inactivation / Initial Denaturation	95°C	01:45	1	-
3	Denaturation	95°C	00:15	45	-
4	Annealing [Data Collection]	62.5°C for BIO-RAD CFX96™ Real-Time PCR Detection System and 62°C for LightCycler® 480 Instrument II Real-Time PCR System	01:00		All Channels

8.2 Fluorescence Dyes

Target name	Reporter	Quencher
SARS-CoV-2	6-FAM & Quasar 670	BHQ-1, BHQ-2
Internal Control	HEX	BHQ-1

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	RdRp gene	6-FAM
	M gene	Quasar 670
Internal Control	IC	HEX

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 Quasar 670. Assign samples to the wells by selecting the appropriate “Sample Type” and afterwards “Load” FAM, HEX and Quasar 670 to the wells. The target name of FAM should be set to “RdRp gene”, the target name of Quasar 670 should be set to “M gene” and the target name of HEX should be set to “IC”.

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 1.0 SARS-CoV-2 Multiplex 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)	Quasar 670 Detection Channel (M)	HEX Detection Channel (Internal Control)
Positive Control	C_t or C_p <40	C_t or C_p <40	C_t or C_p <40
No Template Control (PCR Grade Water)	No C_t or C_p	No C_t or C_p	C_t or C_p <40
Negative Process Control (RNA eluate of known negative)	No C_t or C_p	No C_t or C_p	C_t or C_p <40

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 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).

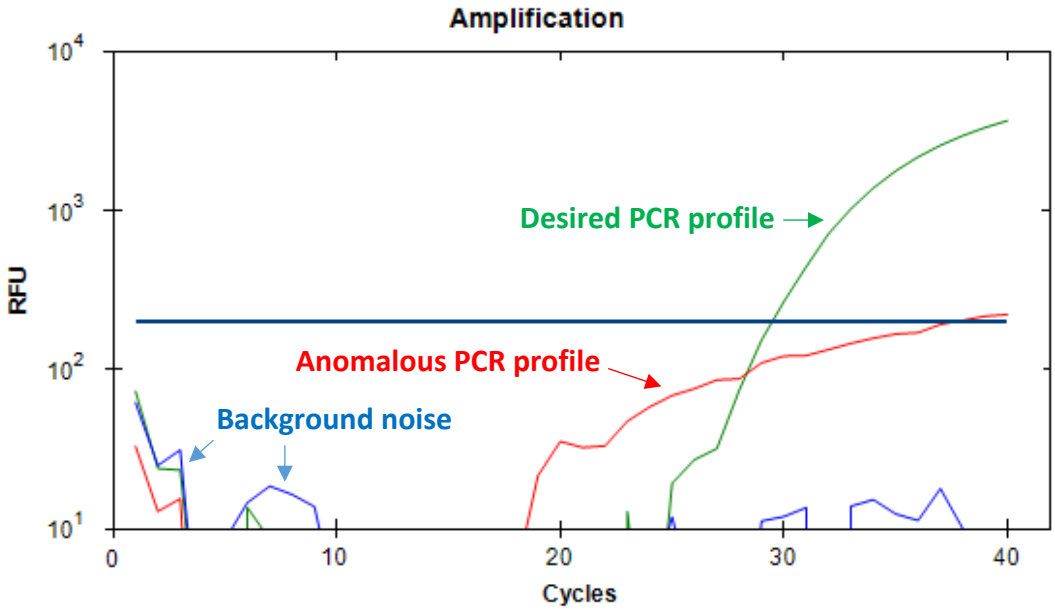
9.2 Interpretation of Results

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)	Quasar 670 (M gene)	HEX (Internal Control)	Result Interpretation
C_t or C_p <40	C_t or C_p <40	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
C_t or C_p <40	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
No C_t or C_p	C_t or C_p <40	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
No C_t or C_p	No C_t or C_p	C_t or C_p < 40	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	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 1.0 SARS-CoV-2 Multiplex 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 200 for all targets.
- Results of the assay should be interpreted in consideration of available clinical information.

10 Performance Evaluation

Performance evaluation of the TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit was done using quantified SARS-CoV-2 RNA spiked into RNA eluate from confirmed negative clinical specimens.

10.1 Analytical Sensitivity

10.1.1 Limit of Detection (LoD)

The experimental design is used to process replicates of dilutions made from three independent samples of known measurand concentrations across 4 days.

Eluates of 30 individual negative patients were obtained and run in duplicates across all testing days.

10.1.1.1 Probit analysis to estimate the LoD

Table 1. Observed Proportions of Positive Test Results.

Copies/Rxn	Sample	Observed Positive Results (out of 20)		Hit Rate	
		RdRP	***M***	***RdRP***	***M***
20	A	20	20	1.000	1.000
	B	20	20	1.000	1.000
	C	20	19	1.000	0.950
15	A	20	20	1.000	1.000
	B	20	18	1.000	0.900
	C	20	19	1.000	0.950
10	A	20	12	1.000	0.600
	B	20	15	1.000	0.750
	C	20	13	1.000	0.650
4	A	18	11	0.900	0.550
	B	13	9	0.650	0.450
	C	17	9	0.850	0.450
2	A	8	4	0.400	0.200
	B	7	4	0.350	0.200
	C	11	5	0.550	0.250
1	A	7	3	0.350	0.150
	B	8	4	0.400	0.200
	C	8	5	0.400	0.250

10.1.1.1.1 RdRP gene

No lack of fit of the probit model was detected using both a Pearson chi-square test and a log-likelihood ratio chi-square test, which allowed prediction of the LoD.

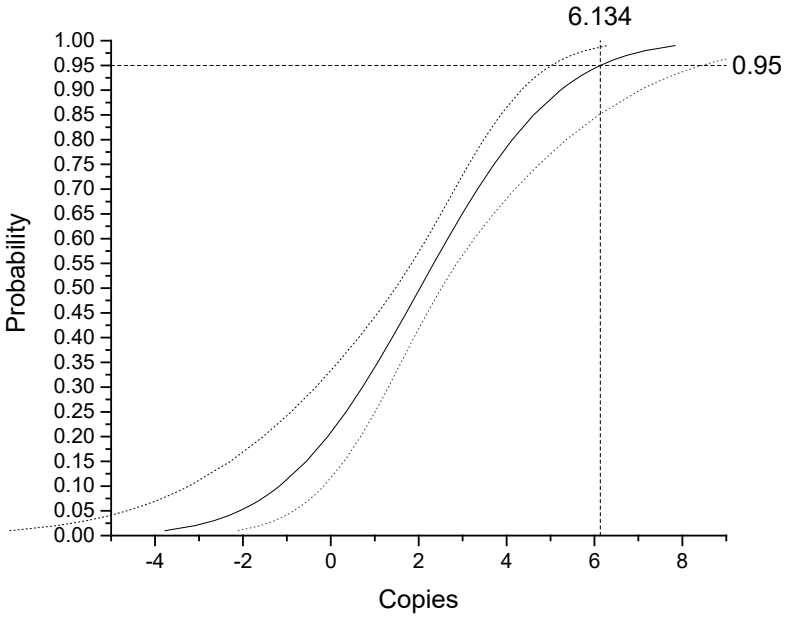


Figure 1. Probit analysis for SARS-CoV-2 *RdRP* gene detection for reagent lot 1 with 95% confidence bounds for the fit.

An estimate of 6.1 copies/reaction is reported as the LoD for the measurement procedure.

10.1.1.1.2 M gene

No lack of fit of the probit model was detected using both a Pearson chi-square test and a log-likelihood ratio chi-square test, which allowed prediction of the LoD.

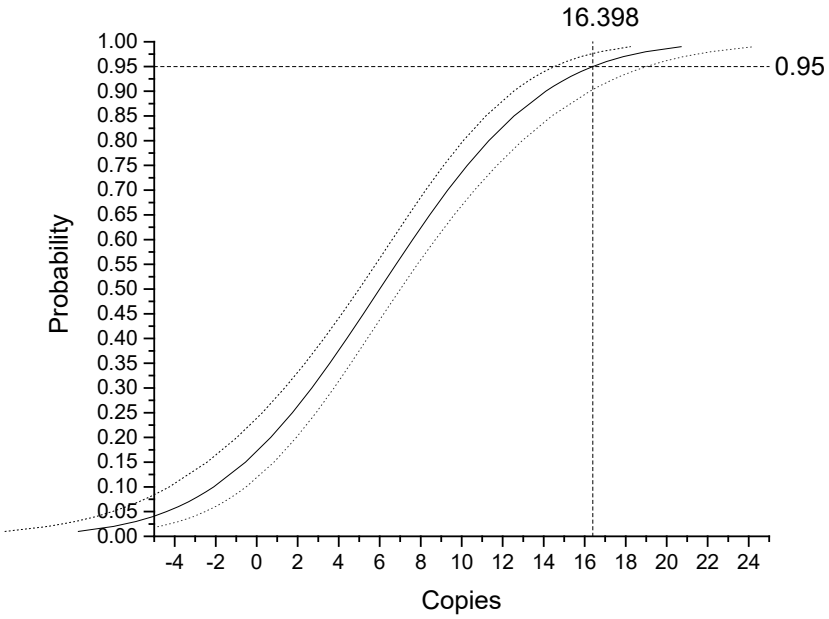


Figure 2. Probit analysis for SARS-CoV-2 *M* gene detection for reagent lot 1 with 95% confidence bounds for the fit.

An estimate of 16.4 copies/reaction is reported as the LoD for the measurement procedure.

10.2 Inclusivity

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

Table 4. Inclusivity – *In silico* analysis for 71759 whole genome sequences of SARS-CoV-2 published via GISAID e.V. (www.gisaid.org) as of 6 August 2020 for the *RdRP* and *M* gene targets: TEPAT 1.0 SARS-CoV-2 RT-PCR Kit.

Whole Genome Sequences		Homology	Comment
RdRP gene	Forward Primer	100%	71467 sequences: 100% identity 93 sequences: 100% identity with at least one degenerate base labels (all synonymous) 17 sequences: 1 base mismatch 1 sequence: 2 bases deletion 1 sequence: Empty sequence for region
	Reverse Primer	100%	71397 sequences: 100% identity 124 sequences: 100% identity with at least one degenerate base labels (all synonymous) 54 sequences: 1 base mismatch 2 sequences: Sequencing deletions in region 2 sequences: Empty sequence for region
	Probe	100%	71461 sequences: 100% identity 98 sequences: 100% identity with at least one degenerate base labels (all synonymous) 17 sequences: 1 base mismatch 2 sequences: Sequencing deletions in region 1 sequence: Empty sequence for region
M gene	Forward Primer	100%	71305 sequences: 100% identity 186 sequences: 100% identity with at least one degenerate base labels (all synonymous) 88 sequences: 1 base mismatch
	Reverse Primer	100%	71162 sequences: 100% identity 251 sequences: 100% identity with at least one degenerate base labels (all synonymous) 164 sequences: 1 base mismatch 2 sequences: 2 base mismatches
	Probe	100%	71167 sequences: 100% identity 295 sequences: 100% identity with at least one degenerate base labels (all synonymous) 116 sequences: 1 mismatch 1 sequence: 4 base mismatches

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 92 sequences do not share 100% identity with the *RdRP* gene assay, and a total of 367 sequences do not share 100% identity with the *M* gene assay. Significantly, all 71759 sequences have 100% identity with either the *RdRP* or *M* gene target. Taken together, reactivity of the specific oligonucleotides included in the TEPAT SARS-CoV-2 Multiplex RT-PCR Kit towards SARS-CoV-2 RNA detection is not expected to be affected.

10.2.2 *In silico* analysis on all SARS-CoV-2 sequences published on NCBI database

Table 5. Inclusivity – *In silico* analysis for 10334 whole genome sequences of SARS-CoV-2 published via NCBI (www.ncbi.nlm.nih.gov) as of 10 August 2020 for the *RdRP* and *M* gene targets: TEPAT 1.0 SARS-CoV-2 Multiplex RT-PCR Kit.

Whole Genome Sequences		Homology	Comment
RdRP gene	Forward Primer	100%	10327 sequences: 100% identity 6 sequences: 100% identity with at least one degenerate base labels (all synonymous) 1 sequence: 1 mismatch
	Reverse Primer	100%	10310 sequences: 100% identity 13 sequences: 100% identity with at least one degenerate base labels (all synonymous) 7 sequences: 1 mismatch 4 sequences: sequencing deletions in region
	Probe	100%	10323 sequences: 100% identity 9 sequences: 100% identity with at least one degenerate base labels (all synonymous) 1 sequence: 1 mismatch 1 sequence: sequencing deletions in region
M gene	Forward Primer	100%	10328 sequences: 100% identity 6 sequences: 1 mismatch
	Reverse Primer	100%	10325 sequences: 100% identity 2 sequences: 100% identity with at least one degenerate base labels (all synonymous) 7 sequences: 1 mismatch
	Probe	100%	10310 sequences: 100% identity 4 sequences: 100% identity with at least one degenerate base labels (all synonymous) 19 sequences: 1 mismatch 1 sequence: 4 mismatches

From the analysis, a single oligonucleotide sequence mutation events leading to < 2 mismatches will not have any significant negative impact on the amplification of the respective target sequences.

A total of 13 sequences do not share 100% identity with the *RdRP* gene assay, and a total of 33 sequences do not share 100% identity with the *M* gene assay. Significantly, all 10334 sequences have 100% identity with either the *RdRP* or *M*

gene targets. Taken together, reactivity of the specific oligonucleotides included in the TEPAT SARS-CoV-2 Multiplex RT-PCR Kit towards SARS-CoV-2 RNA detection is not expected to be affected.

10.3 Limit of Blank (LoB)

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

Table 6. Results of RT-PCR of 30 unique negative patient samples run in duplicates.

Unique Negative Patient Samples					
Specimen	Replicate	Positive/Negative	Ct (FAM™)	Ct (Quasar 670)	Ct (HEX)
1	1	Neg	N/A	N/A	34.83
	2	Neg	N/A	N/A	34.58
2	1	Neg	N/A	N/A	34.95
	2	Neg	N/A	N/A	35.12
3	1	Neg	N/A	N/A	34.68
	2	Neg	N/A	N/A	34.24
4	1	Neg	N/A	N/A	33.05
	2	Neg	N/A	N/A	33.24
5	1	Neg	N/A	N/A	28.02
	2	Neg	N/A	N/A	28.01
6	1	Neg	N/A	N/A	32.91
	2	Neg	N/A	N/A	32.56
7	1	Neg	N/A	N/A	34.35
	2	Neg	N/A	N/A	35.36
8	1	Neg	N/A	N/A	31.11
	2	Neg	N/A	N/A	31.37
9	1	Neg	N/A	N/A	30.65
	2	Neg	N/A	N/A	30.96
10	1	Neg	N/A	N/A	33.99
	2	Neg	N/A	N/A	33.88
11	1	Neg	N/A	N/A	32.00
	2	Neg	N/A	N/A	31.82
12	1	Neg	N/A	N/A	34.06
	2	Neg	N/A	N/A	34.53
13	1	Neg	N/A	N/A	34.58
	2	Neg	N/A	N/A	34.67
14	1	Neg	N/A	N/A	33.02
	2	Neg	N/A	N/A	32.60

15	1	Neg	N/A	N/A	38.15
	2	Neg	N/A	N/A	37.39
16	1	Neg	N/A	N/A	32.04
	2	Neg	N/A	N/A	31.99
17	1	Neg	N/A	N/A	31.95
	2	Neg	N/A	N/A	31.76
18	1	Neg	N/A	N/A	29.97
	2	Neg	N/A	N/A	29.88
19	1	Neg	N/A	N/A	33.03
	2	Neg	N/A	N/A	33.10
20	1	Neg	N/A	N/A	33.13
	2	Neg	N/A	N/A	33.02
21	1	Neg	N/A	N/A	29.55
	2	Neg	N/A	N/A	29.54
22	1	Neg	N/A	N/A	30.64
	2	Neg	N/A	N/A	30.57
23	1	Neg	N/A	N/A	32.38
	2	Neg	N/A	N/A	32.23
24	1	Neg	N/A	N/A	32.70
	2	Neg	N/A	N/A	32.90
25	1	Neg	N/A	N/A	29.66
	2	Neg	N/A	N/A	29.86
26	1	Neg	N/A	N/A	33.81
	2	Neg	N/A	N/A	34.10
27	1	Neg	N/A	N/A	38.49
	2	Neg	N/A	N/A	36.14
28	1	Neg	N/A	N/A	35.36
	2	Neg	N/A	N/A	35.93
29	1	Neg	N/A	N/A	29.55
	2	Neg	N/A	N/A	29.58
30	1	Neg	N/A	N/A	32.00
	2	Neg	N/A	N/A	31.98

A total of 14 replicates of water (no template control (NTC)) reactions was also analyzed over 4 days.

Table 7. Results of RT-PCR of 14 replicates of water as sample input.

Replicates with Water as Sample Input					
Specimen	Replicate	Positive/Negative	Ct (FAM™)	Ct (Quasar 670)	Ct (HEX)
Water (NTC)	1	Neg	N/A	N/A	N/A
	2	Neg	N/A	N/A	N/A
	3	Neg	N/A	N/A	N/A
	4	Neg	N/A	N/A	N/A
	5	Neg	N/A	N/A	N/A
	6	Neg	N/A	N/A	N/A
	7	Neg	N/A	N/A	N/A
	8	Neg	N/A	N/A	N/A
	9	Neg	N/A	N/A	N/A
	10	Neg	N/A	N/A	N/A
	11	Neg	N/A	N/A	N/A
	12	Neg	N/A	N/A	N/A
	13	Neg	N/A	N/A	N/A
	14	Neg	N/A	N/A	N/A

10.4 Analytical Specificity

10.4.1 Cross-reactivity (wet lab testing)

10.4.1.1 Study on other human coronaviruses MERS-CoV and other respiratory pathogens

Table 8. Cross-reactivity studies (wet lab testing).

Pathogen	Strain	Source	Stock Concentration	C_t (FAM™)	C_t (Quasar 670)	C_t (HEX)
MERS-coronavirus	N/A	AMPLIRUN MERS Coronavirus RNA Control	12.500-20.000 copies/µL	N/A	N/A	N/A
Influenza A	H1N1	AMPLIRUN Novel Influenza A H1N1 RNA Control	12.500-20.000 copies/µL	N/A	N/A	N/A
Adenovirus	N/A	AMPLIRUN Adenovirus DNA Control	10.000-20.000 copies/µL	N/A	N/A	N/A
Adenovirus	41	AMPLIRUN Adenovirus DNA Control	10.000-20.000 copies/µL	N/A	N/A	N/A

Note: It is correct that no positive results were obtained from the HEX channel as the controls were not derived from a human specimen, but from purified microbial genomes. The HEX channel is an internal control that only detects the presence of human RNA in the sample.

10.5 Cross-reactivity (*in silico*)

10.5.1 BLAST analysis on the primers/probe

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

1. SARS-coronavirus
2. *Chlamydia pneumoniae*
3. Human coronavirus 229E

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

10.6 Precision

10.6.1 Repeatability/Reproducibility

Low-level samples (20 copies/reaction) of 3 sample lot dilutions were run over three days in 4 PCR runs with 5 replicates per PCR run, amounting to 20 replicates per low-level sample type. Samples were produced by spiking known measurand of RNA standard into pooled negative samples.

Table 9. Precision studies for *RdRP* target.

Reaction	Plate	Sample (20 copies/reaction)	*RdRP*			
			Ct	Mean Ct	SD	% CV
1	1	A	35.51	35.82	0.67	1.87%
2	1	A	35.79			
3	1	A	35.86			
4	1	A	36.25			
5	1	A	37.11			
6	1	B	35.31			
7	1	B	35.66			
8	1	B	35.75			
9	1	B	36.64			
10	1	B	36.71			
11	1	C	34.59			
12	1	C	34.87			
13	1	C	35.53			
14	1	C	35.66			
15	1	C	36.03			
1	2	A	34.77	35.62	0.71	2.01%
2	2	A	35.30			
3	2	A	35.34			
4	2	A	35.63			
5	2	A	36.20			
6	2	B	34.62			
7	2	B	35.51			
8	2	B	35.56			
9	2	B	36.32			
10	2	B	36.48			
11	2	C	34.84			
12	2	C	35.10			
13	2	C	35.24			
14	2	C	36.27			
15	2	C	37.11			

1	3	A	35.14			
2	3	A	35.56			
3	3	A	35.84			
4	3	A	36.05			
5	3	A	36.09			
6	3	B	35.01			
7	3	B	35.15			
8	3	B	35.34	35.74	0.47	1.32%
9	3	B	35.92			
10	3	B	35.98			
11	3	C	35.46			
12	3	C	35.48			
13	3	C	36.17			
14	3	C	36.18			
15	3	C	36.66			
1	4	A	35.55			
2	4	A	35.80			
3	4	A	35.86			
4	4	A	36.04			
5	4	A	37.23			
6	4	B	35.26			
7	4	B	35.30			
8	4	B	35.60	35.86	0.62	1.72%
9	4	B	36.44			
10	4	B	36.55			
11	4	C	34.94			
12	4	C	35.39			
13	4	C	35.57			
14	4	C	35.79			
15	4	C	36.65			

Table 10. Precision studies for *M* target.

Reaction	Plate	Sample (20 copies/reaction)	*M*			
			Ct	Mean Ct	SD	% CV
1	1	A	35.37			
2	1	A	36.18			
3	1	A	36.24			
4	1	A	36.87			
5	1	A	38.07	36.29	0.95	2.61%
6	1	B	35.90			
7	1	B	36.05			
8	1	B	36.14			
9	1	B	36.30			

10	1	B	36.55			
11	1	C	35.12			
12	1	C	35.39			
13	1	C	35.62			
14	1	C	36.05			
15	1	C	38.57			
1	2	A	34.70			
2	2	A	34.84			
3	2	A	35.18			
4	2	A	35.24			
5	2	A	35.43			
6	2	B	34.43			
7	2	B	34.96			
8	2	B	35.50			
9	2	B	35.55			
10	2	B	36.22			
11	2	C	34.97	35.57	1.08	3.03%
12	2	C	35.20			
13	2	C	36.03			
14	2	C	36.45			
15	2	C	38.91			
1	3	A	35.44			
2	3	A	35.78			
3	3	A	35.81			
4	3	A	36.44			
5	3	A	36.47			
6	3	B	34.73			
7	3	B	35.14			
8	3	B	35.42	35.95	0.92	2.55%
9	3	B	35.69			
10	3	B	36.13			
11	3	C	34.78			
12	3	C	36.10			
13	3	C	36.24			
14	3	C	36.61			
15	3	C	38.51			
1	4	A	35.42			
2	4	A	35.94			
3	4	A	35.96			
4	4	A	36.02			
5	4	A	36.37	36.08	0.60	1.67%
6	4	B	35.67			
7	4	B	35.95			
8	4	B	36.36			
9	4	B	36.38			

10	4	B	37.70			
11	4	C	35.21			
12	4	C	35.62			
13	4	C	36.03			
14	4	C	36.55			
15	4	C	N/A			

10.7 Sample Matrix and Nucleic Acid Extraction System Validation

10.7.1 Analytical study on contrived specimens on the claimed specimen type

The LoD and precision studies were all performed on contrived specimens on the claimed sample specimen type. Known measurand of RNA standard was spiked into pooled negative patient eluate. All patient eluates from this study (see sections 1, 3, 4 and 5) were obtained from nasopharyngeal swabs of patients using the Liferiver EX3600 extractions system and the Liferiver Biotech RNA Isolation kit.

10.7.2 Clinical studies on patients' samples

The LoD and precision studies were constructed with five known unique positive patient eluates with 2-3 replicates each.

Table 11. RT-PCR results for unique positive patient samples (not blinded).

Patient	FAM Channel (*RdRP*)				Quasar 670 Channel (*M*)				Hex Channel (Internal Control)			
	Ct	Mean Ct	SD	% CV	Ct	Mean Ct	SD	% CV	Ct	Mean Ct	SD	% CV
1	31.62	31.70	0.106	0.33%	32.69	32.80	0.155	0.47%	25.94	25.92	0.035	0.14%
	31.77				32.91				25.89			
2	25.14	25.21	0.0641	0.25%	26.32	26.39	0.057	0.22%	28.20	28.23	0.030	0.11%
	25.24				26.42				28.26			
	25.26				26.42				28.23			
3	30.22	30.52	0.260	0.85%	31.69	31.94	0.236	0.74%	29.77	29.85	0.098	0.33%
	30.66				31.97				29.96			
	30.68				32.16				29.82			
4	19.41	19.51	0.087	0.45%	21.06	21.22	0.137	0.65%	30.69	30.76	0.133	0.43%
	19.53				21.27				30.67			
	19.58				21.32				30.91			
5	31.09	31.04	0.071	0.23%	31.54	31.68	0.197	0.62%	29.19	29.35	0.219	0.75%
	30.99				31.82				29.50			

Another set of studies was undertaken where positive and negative patient eluates (30 samples each) were blinded to the experimenter.

Table 12. RT-PCR results overview for 60 unique patient samples (blinded).

Patient Sample Type	Positive Call Rate for *RdRP* Target	Positive Call Rate for *M* Target
Positive	30 / 30	30 / 30
Negative	0 / 30	0 / 30

10.8 Instrument Cross-Validation Studies

10.8.1 Verify LoD claim on the different instruments with at least 20 replicates

The LoD claim was independently confirmed on the LightCycler® 480 Instrument II.

Table 13. RT-PCR results overview for 60 low-level samples on the LightCycler® 480 Instrument II.

Sample Concentration (copies/reaction)	Positive Call Rate for *RdRP* Target	Positive Call Rate for *M* Target
20	20 / 20	20 / 20
15	20 / 20	19 / 20
10	19 / 20	11 / 20

LoD of 10 copies/reaction and 15 copies/reaction were confirmed for the detection of SARS-CoV-2 *RdRP* and *M* gene, respectively, on the LightCycler® 480 Instrument II.

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 specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- 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 1.0 SARS-CoV-2 Multiplex 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 1.0 SARS-CoV-2 Multiplex 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:
Email: contact@cellbae.com
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

16 Explanation of Abbreviations

	Abbreviation	Explanation
1	RT-PCR	Reverse transcription polymerase chain reaction
2	RNA	Ribonucleic acid
3	mRNA	messenger RNA
4	SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
5	DNA	Deoxyribonucleic acid
6	cDNA	Complementary DNA
7	IC	Internal control
8	FAM	6-fluorescein amidite
9	HEX	Hexachloro-fluorescein
10	RdRP	RNA-dependent RNA polymerase
11	M gene	Membrane protein gene
12	Ct	Cycle threshold
13	Cp	Crossing point
14	LoD	Limit of detection
15	Rxn	Reaction
16	GISAID	Global initiative on sharing all influenza data
17	NCBI	National Center for Biotechnology Information
18	LoB	Limit of blank
19	NTC	No template control
20	MERS	Middle East respiratory syndrome
21	BLAST	Basic local alignment search tool
22	hMPV	Human metapneumovirus
23	PJP	*Pneumocystis jirovecii*
24	IVD	In-vitro diagnostic