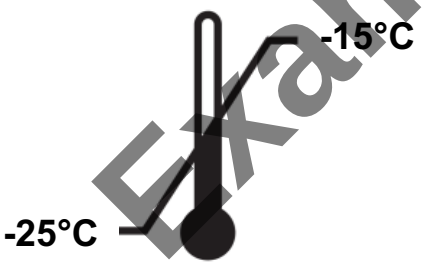
A*STAR FORTITUDE KIT 2.0
COVID-19 Real-Time RT-PCR Test
Instructions for Use

REF **NFIH001** IVD

Σ 200

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

Storage and Transportation Conditions



Protect from light during storage and transportation.

Agency for Science, Technology and Research
SINGAPORE

MiRXES
To Know is to Act

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Product co-developed by A*STAR EDDC, BII, DxD Hub and TTSH

Not available in all countries

Table of Contents

1 Introduction

Purpose: This document describes the use of the A*STAR FORTITUDE KIT 2.0, a real-time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay, for the qualitative detection of SARS-CoV-2 specific RNA in nasal pharyngeal swab samples. The primer probe sets are designed to target a single region specific to SARS-CoV-2. The product is able to run 200 reactions per kit with maximum of 188 patient samples.

Intended Use

The A*STAR FORTITUDE KIT 2.0 is based on real-time Reverse Transcriptase-Polymerase Chain Reaction technology, for the qualitative detection of SARS-CoV-2 specific RNA in nasal pharyngeal swab samples. A positive result from the test may indicate the presence of SARS-CoV-2 specific RNA in the test sample.

Singapore's Health Sciences Authority (HSA) issued a Provisional Authorisation (MDPA2020-01) for this product.

You should not rely on or otherwise use the results as the sole means for clinical diagnosis and treatment. By using A*STAR FORTITUDE KIT 2.0, you agree to the terms and conditions set out in this document.

2 Limitations, Warnings and Precautions

The A*STAR FORTITUDE KIT 2.0 described here have not been systematically verified for platforms or chemicals other than those mentioned in this instructions.

Read the Instructions for Use carefully before using the product.

2.1 Acceptable Specimens

Nasal pharyngeal swab specimens.

2.2 Biosafety Precautions On Specimen Handling

- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Put on protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Discard sample and assay waste according to safety regulation. Obtain the latest guidelines from Singapore's Ministry of Health via https://www.moh.gov.sg/

2.3 Handling the Fortitude Kit 2.0

- Before initial use, check the product and its components for:
  - Cold upon arrival

  - Integrity
  - Completeness with respect to number, type and filling (see section 3)
  - Correct labelling
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time RT-PCR.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the Kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- The workflow in the laboratory should proceed in unidirectional manner. Use separated and segregated working areas for:

  (i) sample preparation,

  (ii) reaction setup and

  (iii) amplification/detection activities.
- Always wear disposable powder-free gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the Kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Do not autoclave reaction tubes after the RT-PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use expired components, refer to the label for expiration date.

3 Product Description

- A*STAR FORTITUDE KIT 2.0 is an *in-vitro* diagnostics (IVD) test kit under Provisional Authorization (MDPA2020-01) issued by Singapore's Health Sciences Authority (HSA).
- A*STAR FORTITUDE KIT 2.0 is based on real-time RT-PCR technology, for the qualitative detection of SARS-CoV-2 specific RNA. The assay includes a positive control and an internal control.
- Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with

fluorescent reporter and quencher dyes.

- The SARS-CoV-2 assay recognizes one single target region on the SARS-CoV-2 viral sequence.
- The probes specific for SARS-CoV-2 RNA are labelled with the fluorophore FAM. The probe specific for Internal Control (IC) is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of SARS-CoV-2 specific RNA, as well as the detection of the Internal Control in corresponding detector channels of the real-time RT-PCR instrument.
- The test consists of three processes in a single assay:
  - Reverse transcription of target RNA to cDNA
  - PCR amplification of target and Internal Control DNA
  - Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

Kit Components

Component Label	Component	Number of Vials	Volume [µL/Vial]
1 (77IC001)	Internal Control template	1	30
2 (113T001)	Positive Control	1	150
3 (BNC001)	Negative Control	1	330
4 (BRM001)	Universal Probes Reaction mix	3	920
5 (BRT001)	Reverse Transcriptase	1	110
6 (FH001)	Primers-Probes Mix	1	220
7 (BNFW001)	Nuclease-Free water	2	1000

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

4 Storage and Transportation

- The A*STAR FORTITUDE KIT 2.0 is shipped in a cold chain environment. The components of the kit should arrive cold. If the kit components are not cold upon receipt, or if vials have been compromised during shipment, contact Technical Support (refer to section 11) for assistance.
- All components are to be stored between -25°C and -15°C upon arrival.
- Protect from light.

5 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 whether using product sources provided or other product sources.*

- Appropriate nasal pharyngeal swabs for biological specimen collection. A negative result from the A*STAR FORTITUDE KIT 2.0 may be a result of failed specimen collection. Interpretation of assay result should take into consideration available clinical information.
- Appropriate nucleic acid extraction kit (see section 6.1):
- The A*STAR FORTUTUDE KIT 2.0 had been used on RNA samples extracted with bioMérieux NucliSens easyMAG system, QIAamp Viral RNA Mini Kit, QIAGEN EZ1 Virus Mini Kit, QIAGEN EZ1 Advanced and QIAGEN EZ1 Advanced XL systems.
- Appropriate real-time PCR instrument (see section 6.4).
- Desktop centrifuge with a rotor for 1.5 and 2 mL reaction tubes.
- Centrifuge that goes up to 1000 x *g* with a rotor for microtiter plates, if using 96-well reaction plates.
- Vortex mixer.
- Appropriate 96-well reaction plates or reaction tubes with corresponding (optical) closing material.
- Pipettes (adjustable)
- Disposable pipette tips with filters and aerosol barriers.
- Disposable powder-free gloves.

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

6 Procedure

6.1 RNA Sample Preparation

- Extracted RNA is the starting material for the A*STAR FORTITUDE KIT 2.0.
- A*STAR FORTITUDE KIT 2.0 has been validated using RNA samples extracted from clinical nasal pharyngeal swab samples via the bioMérieux NucliSens easyMAG automatic total nucleic acid extraction system. Other extraction systems tested include the QIAamp Viral RNA Mini Kit, QIAGEN EZ1 Virus Mini Kit, QIAGEN EZ1 Advanced and QIAGEN EZ1 Advanced XL systems.
- The suitability of the other nucleic acid extraction procedure for use with A*STAR FORTITUDE KIT 2.0 has to be validated by the user.
- The quality of the extracted RNA has a significant impact on the performance of downstream RT-PCR assay. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time RT-PCR technology.
- Users are strongly recommended to include an **extraction control** to monitor

RNA extraction efficiency for the RNA extraction protocol of choice.

- A negative result from the A*STAR FORTITUDE KIT 2.0 may be a result of 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 RT-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 Technical Support (see section 11).

6.2 RT-PCR Reaction Master Mix Preparation

- Do not combine components of assay kits with different lot numbers.
- To prepare the master mix, thaw all reagents and samples on ice completely.
- Add the components, by pipetting, in order of the sequence as shown in the table below, mix the master mix by gentle pipetting or vortexing, and centrifuge briefly before use.

Master Mix Preparation

Sequence	Component Label	Master Mix	Volume per Reaction (µL)
1	7 (BNFW001)	Nuclease-Free water	8.40
2	4 (BRM001)	Universal Probes Reaction mix	12.50
3	6 (FH001)	Primers-Probes Mix	1.00
4	5 (BRT001)	Reverse Transcriptase	0.50
5	1 (77IC001)	Internal Control (IC) template	0.10
--	--	Test Sample (RNA sample/Positive Control/Negative Control)	2.50
		Total	**25.00**

6.3 Reaction Setup

- Do not combine components of assay kits with different lot numbers in the same RT-PCR set-up.
- Pipette 22.5 µL of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

- Add 2.5 µL of the sample (eluate from the nucleic acid extraction) or 2.5 µL of the control (Positive or Negative Control).
- Make sure at least one Positive Control and one Negative Control are used per run.
- Thoroughly mix the test samples or Positive Control or Negative Control template with the Master Mix by gently pipetting up and down.
- Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x *g*.

6.4 Real-Time PCR Instrument

A*STAR FORTITUDE KIT 2.0 was developed and validated to be used with the following instruments:

- BIO-RAD CFX96™ Dx System (Model: 1845097-IVD) and CFX96™ Dx Optical Reaction Module (Model: 1845097-IVD).
- BIO-RAD CFX96™ TOUCH™ Real-Time PCR Detection System (Model: 1855195) and CFX96™ Optics Module.

*A*STAR FORTITUDE KIT 2.0 may not deliver the same results if used with other systems.*

6.5 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 A*STAR FORTITUDE KIT 2.0 on other real-time PCR instruments, please contact Technical Support (see section 11).

6.6 Temperature Profile and Dye Acquisition

Step	Temperature (°C)	Duration (Hr:Min:Sec)	No. of Cycles	Detection
Reverse Transcription	48	00:15:00	1	-
RT inactivation / Initial Denaturation	95	00:02 30	1	-
Denaturation	95	00:00:20	42	-
Annealing [Data Collection]	59	00:00:42		√ All Channels

6.7 Fluorescence Detectors (Dyes)

S/N	Target	Channel
1	SARS-CoV-2	FAM
2	Internal Control	HEX

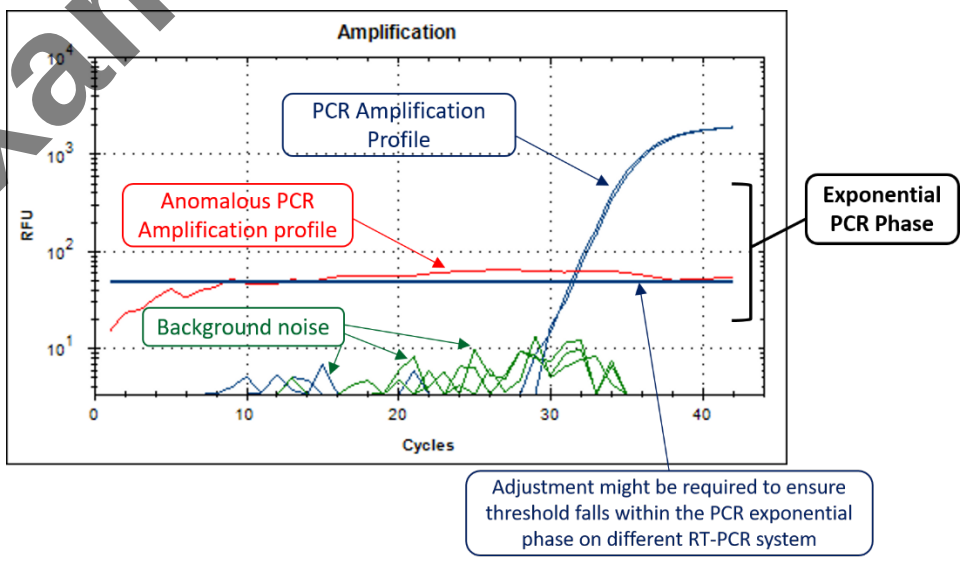
7 Data Analysis

- For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.
- If you are using the BIO-RAD CFX96™ Dx System (Model: 1845097-IVD) and CFX96™ Dx Optical Reaction Module (Model: 1845097-IVD) or the BIO-RAD CFX96 TOUCH™ Real-Time PCR Detection System (Model: 1855195) and CFX96™ Optics Module, please refer to the recommendation below before data analysis.

C_T Determination By Single Threadshold

- The recommended threshold for this assay on the above described real-time PCR systems is **50** for both FAM and HEX channels, based on a Relative Fluorescence Units (RFU) range of 0-2000 for the FAM channel and RFU range of 0-1250 for the HEX channel.
- For potential instrument-to-instrument variation in the fluorescence signal saturation range, instrument calibration might be required to set the appropriate threshold for individual real-time PCR system.
- *All C_T values reported in this IFU is based on the recommended threshold settings at 50 for both FAM and HEX channels as described above. C_T values are subject to changes with different thresholds set by the users.*
- **Interpretataion of A*STAR FORTITUDE KIT 2.0 test result** should take into consideration **the C_T values,** as well as **the shape of the amplification curve**, as shown in Figure 1 below.

Figure 1. Amplification Plot Window.



Note:

(1) Anomalous PCR amplification profile in red is signal due to non-specific amplification. C_T values obtained from such amplification curves are not valid and

should not be taken into consideration in data analysis and result interpretation. (2) PCR amplification profile in blue shows examples of fluorescence growth curves as a result of specific amplification. Only C_T values obtained from such amplification curves are valid and should be taken into consideration in data analysis and result interpretation.

C_T Determination By Regression

- An alternative option for the analysis of data from the PCR platforms described above is the use of regression mode for C_T determination. Details of this can be found in the user manuals of the respective instrument.

- For detailed instructions regarding the analysis of the data generated with the A*STAR FORTITUDE KIT 2.0 on different real-time PCR instruments please contact Technical Support (see section 11).

7.1 Interpretation of Test Results

Sample	SARS-CoV-2 (FAM)	IC (HEX)	Interpretation
Positive Control (PC)	1. C_T values < 40[a] , AND 2. Presence of fluorescence growth curves in the PCR amplification step.	C_T values < 40[a]	QC passed
Negative Control (NC)	1. C_T undetermined; AND 2. Absence of fluorescence growth curves in the PCR amplification step.	C_T values < 40[a]	
Test Sample	1. C_T < 40; AND 2. Presence of fluorescence growth curves in the PCR amplification step.	C_T values < 40[a], or C_T undetermined[b]	Positive for SARS-CoV-2
	1. C_T undetermined; AND 2. Absence of sigmoidal fluorescence growth curves in the PCR amplification step.	C_T values < 40[a]	Negative for SARS-CoV-2
	C_T undetermined	C_T : undetermined	Invalid Test Run

a – Note that C_T values for PC and IC are about 32 ± 1 C_T, based on the recommended threshold setting at 50 as described above. Marked deviation from the these values should be queried before releasing results.
b – However, failture to detect IC should be queried in the event of positive signal for SARS-CoV-2 detection channel.

- If a false positive occurs with one or more NC reactions, sample contamination may have occurred.
- In case of an **invalid** test run, repeat testing with strict adherence to the procedure guidelines, using the remaining purified nucleic acids or start from the residual biological specimen again.
- As per standard RT-PCR data analysis, interpretation of A*STAR FORTITUDE KIT 2.0 test result should take into consideration the **C_T values**,

as well as the **fluorescence growth curves** in the PCR amplification step as shown in Figure 1.

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

8 Performance Evaluation

Performance evaluation of the A*STAR FORTITUDE KIT 2.0 was done using quantified SARS-CoV-2 DNA (SARS-CoV-2 – FAM), internal control templates (IC – HEX) and primer-probes.

Parameter	Specifications
Limit of Detection	25 copies/reaction
Cross-Reactivity	Do not cross react with SARS-CoV, Flu A pH1N1, Flu A H3N2, Flu B and MERS-CoV.
Linearity	10 – 2.5 x 10^5 copies/reaction

8.1 Analytical Sensitivity

The limit of detection (LoD) was determined to be 25 copies/reaction, with assay efficiency at 99% and an R^2 value of 0.99, via a dilution series of 250,000 copies/reaction to 10 copies/reaction.

8.2 Analytical Specificity

A*STAR FORTITUDE KIT 2.0 did not cross-react with any of the following pathogens: SARS-CoV, Flu A pH1N1, Flu A H3N2, Flu B and MERS-CoV, following the test procedues described in section 6 and section 7.

Well	Sample	SARS-CoV-2 (FAM)			IC (HEX)		
		C_T[e]	Ave	Std Dev	C_T[e]	Ave	Std Dev
A09	SARS-CoV[d] (100copies)	(N/A[c])	(N/A[c])	N/A	32	31	0.37
A10		N/A			31		
A11	SARS-CoV (50copies)	N/A	N/A	N/A	31	31	0.21
A12		N/A			31		
B11	Flu A pH1N1[d] (10^3 copies)	N/A	N/A	N/A	31	31	0.15
B12		N/A			31		
C11	Flu A H3N2[d] (10^3 copies)	N/A	N/A	N/A	32	31	0.31
C12		N/A			31		
D11	FLU B[d] (10^3 copies)	N/A	N/A	N/A	31	32	0.42
D12		N/A			32		
E11	MERS-CoV[d] (10^4 copies)	N/A	N/A	N/A	32	31	0.29
E12		N/A			31		
F11	PC (250 copies)	31	31	0	31	31	0.09
F12		31			31		
H11	NC	N/A	N/A	N/A	32	31	0.24
H12		N/A			31		

c – Note that data analysis showed C_T value of 14 at well A09. However, this was due

to non-specific signal based on shape of the amplification curve (the example of anomalous PCR amplification profile shown in Figure 1 above was the non-specific amplification curve associated with this datapoint). Data as such is considered invalid, as explained in section 7.1, therefore "N/A" is assigned in place of the C_T value of 14, which is an invalid C_T value.
d – SARS-CoV, Flu A pH1N1, H3N2, FluB and MERS-CoV RNA are WHO RNA standard.
e – C_T values reported above are based on the recommended threshold setting at 50 as described in section 7 above.

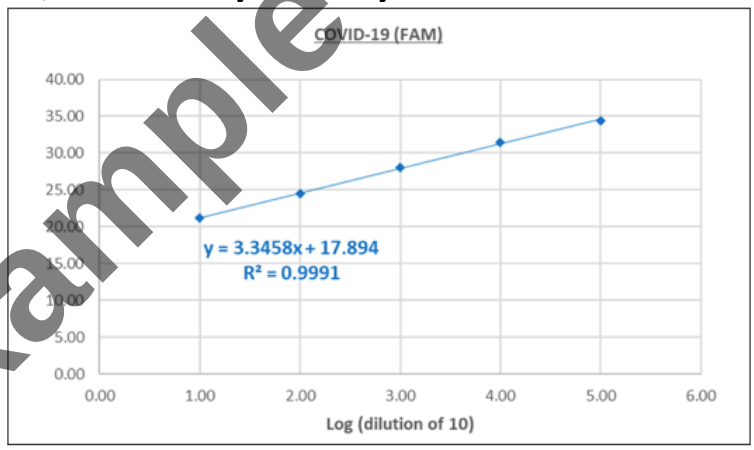
8.3 Repeatability and reproducibility

Operator	Sample	SARS-CoV-2 (FAM)			IC (HEX)		
		Ave	Std Dev	%CV	Ave	Std Dev	%CV
1	PC	32	0.17	0.55%	31	0.13	0.41%
	NC	N/A	N/A	N/A	31	0.22	0.69%
2	PC	32	0.15	0.47%	32	0.46	1.43%
	NC	N/A	N/A	N/A	31	0.32	1.01%

Note that C_T values reported above are based on the recommended threshold setting at 50 as described in section 7 above.

8.4 Linearity

The linear range was determined to be 10 copies/reaction to 250,000 copies/reaction, with an assay efficiency of 99% and an R^2 value of 0.99.



9 Assay 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, including testing procedures and interpretation of results prior to performing the assay.
- 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 impurity 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 A*STAR FORTITUDE KIT 2.0 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 mis-mataches 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.

10 Quality Control

- To ensure consistent product quality, each lot of the A*STAR FORTITUDE KIT 2.0 is tested against predetermined specifications.
- Users are strongly discouraged to combine components from assay kits of different lot numbers.

11 Technical Assistance

For technical advice, please contact Technical Support:

COVID19@accelerate.tech

12 Disclaimers

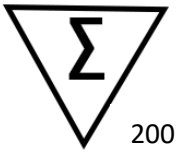
A*STAR FORTITUDE KIT 2.0 should only be used for the intended purpose and in accordance with the Instructions for Use.

Accelerate Technologies Pte Ltd (DxD Hub) is not liable for any damage or loss that may result from your use of the test.

13 Explanation of Symbols and Abbreviations

Catalogue number

In-Vitro Diagnostics



Maximum 200 reactions

A*STAR – Agency for Science, Technology and Research

A*STAR EDDC – Experimental Drug Development Centre

A*STAR BII – Bioinformatics Institute

DxD Hub – Diagnostics Development Hub

TTSH – Tan Tock Seng Hospital

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