CE

PHASiFY™ DeCOVID

SARS-CoV-2 RT-qPCR Kit

Multiplex real-time RT-qPCR test intended for qualitative detection of nucleic acid from SARS-CoV-2

100 reactions

USER MANUAL

IVD

REF 3010300

PHASE Scientific Int'l Ltd.

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Contents

Product Introduction 1
Intended Use 2
Summary and Explanation of the Test 3
Kit Composition 4
Contents of Kit 5
Equipment & Reagents Not Included 5
Storage Conditions 6
Precaution & Handling Requirement 7
Sample Collection, Transport & Storage 8
RNA Extraction 8
Reagent Preparation and PCR Setup 9
Protocol 10
Results Interpretation 11
Limitations 13
Quality Control 14
Technical Assistance 14
Troubleshooting 15
Frequently Asked Questions (FAQs) 16
Performance Charateristics 17-25
Symbols 26

PRODUCT INTRODUCTION

PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit is a multiplex reverse transcription qPCR assay (RT-qPCR) for detection of SARS-CoV-2 RNA extracted from common human respiratory tract specimens. The test uses RT-qPCR and fluorescent probe-based detection chemistry to detect two specific genes in the ORF1b region of SARS-CoV-2 genome. The kit contains enough reagents for 100 reactions that includes positive and negative controls to validate test results.

Sample Input	Number of Reactions
Up to 10 µL of extracted RNA from clinical specimen	100

INTENDED USE

The PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit is intended for qualitative detection of nucleic acid from SARS-CoV-2 in common human respiratory tract specimens. This test is for in-vitro diagnostic use, and is intended to be used by clinical or laboratory personnel specifically trained in the procedures and proper handling of reagents/materials for RT-qPCR reaction set up and RT-qPCR machine operation.

Results are for the identification of SARS-CoV-2 RNA which is generally detected in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

SUMMARY & EXPLANATION OF THE TEST

Explanation of the Test

The PHASIFY™ DeCOVID RT-qPCR Kit is a multiplex RT-qPCR assay consisting of three primer and probe sets in a single reaction detecting two specific viral targets (Orf1b and RdRP gene of SARS-CoV-2 genome) and one internal control (Human RNase P Protein gene), along with enhanced throughput and ease of use as the advantages of a multiplex molecular detection assay. This kit was validated using human respiratory tract samples.

Principle of the Test

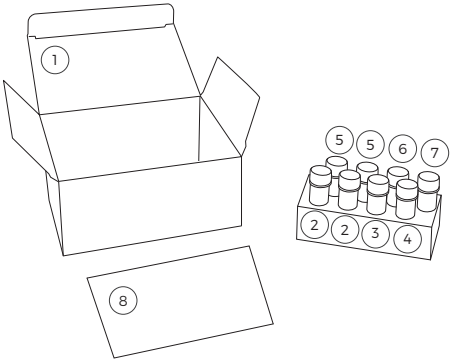
The PHASIFY™ DeCOVID RT-qPCR Kit utilizes probe-based chemistry to discriminate selective amplification of the three detection targets. The fluorescence reporter of ORF1b, RdRP and RNase P probes are FAM, HEX and TAMRA with BHQ1 as quencher respectively.

Upon successful RNA extraction from clinical samples, selective amplification of target nucleic acid is achieved by using target-specific forward and reverse primer and probe specific to ORF1b and RdRP viral genes.

Selective amplification of Internal Control is achieved by using non-competitive, sequence specific forward and reverse primers and a probe which have no homology with the coronavirus genome.

KIT COMPOSITION

Ref. No. 3010300



PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit (100 reactions)

1. Packaging Box
2. 2x Reaction Buffer
3. Enzyme Mix
4. Primer Probe Mix
5. Nuclease free water
6. No Template Control
7. Positive Template Control
8. Product Insert

CONTENTS OF KIT PDP101

Content	Quantity	Storage
Nuclease free water	2 x 800 µL	-20°C or below
2x Reaction Buffer	2 x 800 µL	
Enzyme Mix	160 µL	
Primer Probe Mix	240 µg (powder)	
No Template Control	800 µL	
Positive Template Control	36.2 pg (powder)	

EQUIPMENT & REAGENTS NOT INCLUDED

In addition to the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit, the following items are required:

Equipment & Materials
RNA Extraction Kit
qPCR Thermocycler
Microcentrifuge (adjustable, up to 13,000 x g)
Adjustable pipettes (10, 20, 100, 200, 1000 µL)
Sterile, RNase-free pipette tips with aerosol barrier
Vortex-mixer
Microcentrifuge tubes (0.5 mL and 1.5 mL)
8 well PCR tube strips/ 96-well PCR plate
Mini-spin Centrifuge
Cold block(s) or ice

STORAGE CONDITIONS

All components should be stored at -20°C or below and avoid repeated freeze and thaw cycles. Protect the primer and probe mix from light as prolonged exposure can diminish the activity of the fluorophores. Keep reagents separate from sample material to avoid contamination.

Refer to individual components for stated expiry and storage conditions.

PRECAUTION & HANDLING REQUIREMENT

- Positive results indicate presence of SARS-CoV-2 RNA only
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures
- The use of sterile and nuclease-free pipette tips is recommended
- Use only supplied or specified consumables to ensure opitmal test performance
- Safety Data Sheets (SDS) are available on request
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly
- Any deviation from the procedures and guidelines may affect optimal test performance
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing
- If not tested immediately, store extracted RNA at ≤ -70°C until use and keep on ice during testing
- Wear disposable gloves and change regularly or immediately if there is potential contamination
- Waste should be disposed of in accordance with applicable local, state/provincial, and/or national regulation

SAMPLE COLLECTION, TRANSPORT & STORAGE

The PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit is intended to be used with RNA extracted from common human respiratory tract specimens.

All samples and controls should be handled as if they are capable of transmitting infectious agents.

RNA EXTRACTION

RNA should be extracted from human respiratory tract samples using a compatible RNA extraction process. Most standard viral RNA extraction kits should be compatible with this assay. The sample type (nasal, sputum, throat, etc.), viral RNA extraction kit, and extraction process should be validated by the user.

The following RNA extraction kits were tested and are compatible with the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit:

- PHASIFY VIRAL RNA Extraction Kit
- QIAamp Viral RNA Mini Kit

Please perform the RNA extraction on samples following the manufacturer's instructions for use.

REAGENT PREPARATION AND PCR SETUP

When using the kit for the first time, prepare:

- Primer Probe Mix:
  Add 375 µL nuclease free water to the Primer Probe Mix tube. Vortex to ensure the powder is dissolved well. Incubate on ice.
- Positive Template Control:
  Add 200 µL nuclease free water to the Positive Template Control tube. Vortex to ensure the powder is dissolved well. Incubate on ice.

Note: Be careful not to contaminate the environment or other reagents by the Positive Template Control.

PCR Cycler Set-up

1. Select Standard Curve for the Experiment type.
2. Select TaqMan® Reagents for the Chemistry.
3. Select any run mode. Fast mode is preferred.
4. Set up the signal detection channels for different targets as follow:

Target	Fluorescence signal (Absorbtion / emission wavelength)	Quencher
ORF1b	FAM (494 nm / 525 nm)	None
RdRP	VIC/HEX (535 nm / 554 nm)	None
RNase P	TAMRA (565 nm / 580 nm)	None

5. Set the Passive Reference Dye as None (if available).
6. Run the thermal cycling program as below:

Temperature	Time	Number of cycles
55°C	15 min	1
95°C	30 sec	1
95°C	10 sec	45
63°C	30 sec	
4°C (optional)	∞	1

The fluorescence signal is collected every cycle at 63°C.

PROTOCOL

1. Thaw all reagents to be used in the run on ice.
2. Always briefly spin the thawed reagent tubes before opening to prevent any reagent trapped on the cap.
3. Prepare RT-qPCR master mix for the number of samples (n) to be tested according to table below:

Reagent	Vol for a single rxn (µL)	Vol for n samples + 2 controls (µL)
2x Reaction Buffer	15	15 x (n+3)
Enzyme Mix	1.5	1.5 x (n+3)
Primer Probe Mix	3.5	3.5 x (n+3)

4. Create a map of the sample and control well layout.
5. Pipette 20 µL of RT-qPCR master mix prepared above into each of the PCR reaction wells as per the map.
6. Add 10 µL of RNA extracted from clinical samples to be tested into the sample wells. Mix well by pipetting up and down.
7. Add 10 µL of **"No Template Control"** into the negative control well. Mix well by pipetting up and down.
8. Add 10 µL of **"Positive Template Control"** into the positive control well. Mix well by pipetting up and down.

   NOTE: Be careful not to contaminate the environment or other reagents by the Positive Template Control.
9. Seal the PCR well tube tightly and spin down the reaction mix to the bottom of the well.
10. Place the PCR well tube in the RT-qPCR instrument and run the thermal cycling program.

RESULTS INTERPRETATION

1. Manually adjust the thresholds of FAM, VIC/HEX and TAMRA channel to the exponential phase of the fluorescence curve and above the signal from NTC (noise).

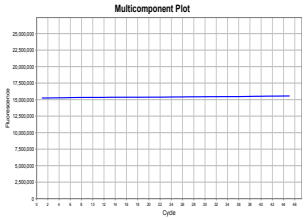
2. Examine the positive control and NTC for their Ct value. An exponential amplification curve should be observed in all channels for the positive control. If the controls results are not valid, the sample results cannot be interpreted.

Control	Channel	Acceptable C_t
Negative Control	FAM and VIC/HEX	≥ 40
	TAMRA	≥ 35
Positive Control	FAM and VIC/HEX	< 38
	TAMRA	< 35

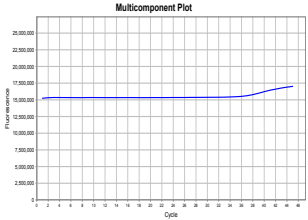
3. When assessing the sample results, carefully examine the amplification curves of the samples. Only true exponential curves should be interpreted as positive.

NOTE: Multi-component plot or raw fluorescence data would be very useful to help distinguishing between weak positive and negative sample.

Negative



Weak Positive



Negative signal should have a flat multicomponent plot during the entire qPCR cycling whereas weak positive signal should have visible surge in fluoresence intensity in the late cycle.

Interpretation of Results

FAM	HEX	TAMRA	Result
C_t < 40	C_t < 40	Any C_t	SARS-CoV-2-positive
C_t < 40	C_t ≥ 40	Any C_t	SARS positive, regard as SARS-CoV-2 presumptive positive due to low prevalence of SARS-CoV
C_t ≥ 40	C_t < 40	Any C_t	SARS-CoV-2-positive
C_t ≥ 40	C_t ≥ 40	C_t ≤ 35	SARS-CoV-2 negative
C_t ≥ 40	C_t ≥ 40	C_t > 35	Invalid test

LIMITATIONS

- For reliable results, it is essential to adhere to the guidelines given in this manual. Changes in reaction setup or cycling protocol may lead to failed experiments.
- Spontaneous mutations within the target sequence may result in failure to detect the target sequence. While this risk is mitigated in the test's design, if failure to detect the target is expected, it is recommended to test the specimen with a different test that detects different target sequences from the SARS-CoV-2 genome.
- Based on the in silico analysis, SARS-CoV may cross react with the ORF1b primer sets of the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- Results must always be interpreted in consideration of all other data gathered from a sample. Interpretation must be performed by personnel trained and experienced with this kind of experiment.
- Users should be trained to perform this assay and competency should be documented.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- False negative result may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

QUALITY CONTROL

PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit is produced in accordance with PHASE Scientific's Quality Management System. Each lot is tested against predetermined specifications to ensure consistent product quality.

TECHNICAL ASSISTANCE

If you have any queries regarding PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit, please do not hesitate to contact us by:

Email: phasify@phasesci.com
Service hotline: +(852) 3700 8888

The PHASE Technical Service Team will help to solve your concerns with our best effort.

TROUBLESHOOTING

Observation	Possible cause	Corrective measures
Amplification in negative control	· Contamination during RNA extraction or PCR plate setting	· Decontaminate the working area with 10% bleach, water and 70% ethanol · Repeat the RNA extraction or PCR plate set-up using new and fresh reagents
C_t value is detected but multicomponent plot indicates no amplification	· The threshold is not set appropriately	· Manually adjust the threshold above the noise level of the false positive signal
	· Non-linear / drift baseline	· The C_t value is invalid and regarded as no signal detection ($C_t \geq 40$)
Weak to no signal in positive control	· Incorrect PCR program setting	· Following the guideline strictly to set up the PCR run
	· The positive control is not mixed properly after thawing	· Make sure to vortex mix the positive control after thawing
	· Pipetting errors when loading the positive control	· Make sure 10 µL of positive control is being pipetting up before dispensing
	· Components within the kit are degrading due to improper storage conditions	· Following the storage condition recommended in this IFU strictly
	· The kit may have expired	· Discard the kit if expired

FREQUENTLY ASKED QUESTIONS (FAQs)

What if I have less than 10 µL RNA sample?

If less than 10 µL RNA will be analysed, the sample volume should be topped up to 10 µL with ultra-pure water.

Which RNA extraction kits are compatible to the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit?

The following RNA extraction kits were tested and are compatible with the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit:

- PHASIFY VIRAL RNA Extraction Kit
- QIAamp Viral RNA Mini Kit

Which real time PCR machines are compatible to PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit?

The following qPCR machines were tested and are compatible with the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit:

- QuantStudio™ 3 Real-Time PCR System
- ViiA™ 7 Real-Time PCR System
- CFX96 Touch Real-Time PCR Detection System

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Limit of Detection)

A two-phase study was performed to evaluate the Limit of Detection (LoD) of the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit using heat-inactivated SARS-CoV-2 virus from ATCC (Cat# VR-1986HK™). The genomic copies concentrations of the heat inactivated SARS-CoV-2 was be predetermined by ddPCR after extraction by QIAamp Viral RNA Mini Kit. Pooled nasopharyngeal swab specimens were spiked with different concentration of heat-inactivated SARS-CoV-2 (genomic copies (cp)/mL). The spiked nasopharyngeal swab specimens were extracted by QIAamp Viral RNA mini kit and measured on the QuantiStudio 3 instrument according to the Instructions for Use protocol. In the first phase, the preliminary LoD was established by testing three replicates per each concentration level: 50 cp/mL, 100 cp/mL, 200 cp/mL, 500 cp/mL, 1000 cp/mL and 2000 cp/mL in nasopharyngeal swab specimens. The tentative LoD was determined to be 100 cp/mL.

The Limit of Detection (LoD) is defined as the lowest concentration of SARS-CoV-2 (genomic copies/mL) that can be detected with a probability of 95% or greater. In the second phase, the preliminary LoD was then confirmed by testing 20 extraction replicates of nasopharyngeal swab specimens spiked with specific amount of heat-inactivated SARS-CoV-2 virus. The study results are summarized at Table 1 and the LoD was determined to be 200 cp/mL for nasopharyngeal specimens using QIAamp Viral RNA mini kit.

Table 1. LoD confirmatory study results of PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit

Target Level	Valid Results	Mean Ct			Positive Detection Rate
		ORF1b	RdRP	Rnase P	
200 cp/mL	20	35.0	35.9	25.8	100% (20/20)
100 cp/mL	20	37.7	37.6	26.3	50% (10/20)

Inclusivity (Analytical Reactivity)

The inclusivity of the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit was evaluated using in silico analysis of the assay primers and probes on 52179 of SARS-CoV-2 genome sequences available on GISAIS database on 18 August 2020. The ORF1b assay exhibits 100% homology with over 99% of the SARS-CoV-2 genomes, while most of the genomes with identified mismatches are not expected to harm PCR performance either evidenced by literature reports or because of degenerate base codes alignment. The reverse primer of RdRP assay shows a single mismatch to all aligned genomes, but the mismatch type is proven to have no or minimal influence to PCR performance. Therefore, the RdRP assays can also detect over 99.9% of the SARS-CoV-2 genomes. Critical mismatches on either ORF1b or RdRP assay could be identified in a few (6 out of 52179) genomes. However, the occurrence is very low and none of the concerned genomes exhibits critical mismatch in both assays. Therefore, the concerned genomes will correctly return positive results as well from these rare viral genome variants. In conclusion, all of the published SARS-CoV-2 sequence exhibited no mismatch, or with mismatch which can be justified to prove it will not affect the detection of the SARS-CoV-2 strains by the PHASIFY™ DeCOVID RT-qPCR Kit. The result shows that PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit can detect all strains of currently identified SARS-CoV-2.

Cross-Reactivity (Analytical Specificity)

The cross-reactivity of the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit was evaluated in silico by comparing the primer and probe sequences against the related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in a clinical specimen. The result showed that PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit has low cross reactivity to all of the 26 pathogens in Table 2 except SARS-CoV. Although the >80% homology between some of the SARS-CoV genomes and primer/probe sequences in the assays shows that the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit may cross-react with some of the SARS-CoV genomes. Considering the low prevalence of SARS-CoV in current society, the risk of false positive signal from SARS-CoV is very low and thus the result demonstrates the high specificity of PHASIFY™ DeCOVID to detecting predominately SARS-CoV-2.

Table 2. In Silico Analysis Result for the Cross-reactivity

Organism	RdRP					Orf1b			RNaseP		
	Forward Primer (R=G)	Forward Primer (R=A)	Reverse Primer (W=A)	Reverse Primer (W=T)	Probe	Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
Human coronavirus 229E		≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human coronavirus OC43	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human coronavirus HKU1	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human coronavirus NL63	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
SARS-coronavirus	90%	100%	100%	96%	100%	100%	100%	100%	≤80%	≤80%	≤80%
MERS-coronavirus	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Adenovirus (e.g. C1 Ad. 71)	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	83%
Human Metapneumo-virus (hMPV)	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%

Parainfluenza virus 1	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Parainfluenza virus 2	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Parainfluenza virus 3	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Parainfluenza virus 4	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Influenza A	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Influenza B	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Enterovirus and Rhinovirus	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Respiratory syncytial virus	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Chlamydia pneumoniae	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Haemophilus influenzae	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Legionella pneumophila	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Mycobacterium tuberculosis	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	Not found	≤80%	≤80%	≤80%	≤80%
Streptococcus pyogenes	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Bordetella pertussis	≤80%	Not found	Not found	Not found	≤80%	Not found	Not found	Not found	≤80%	Not found	≤80%
Mycoplasma pneumoniae	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Pneumocystis jirovecii (PJP)	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Candida albicans	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Pseudomonas aeruginosa	≤80%	≤80%	≤80%	≤80%	Not found	Not found	≤80%	≤80%	≤80%	≤80%	≤80%
Staphylococcus epidermis	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	86%	≤80%	≤80%	≤80%	≤80%
Streptococcus salivarius	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%

Interference Substance

The interference effect of substances that may potentially be found in respiratory specimens on the performance of PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit were evaluated. SARS-CoV-2 negative nasopharyngeal swab specimens were spiked with or without heat-inactivated SARS-CoV-2 to a final concentration of 3X LOD. Individual interference substance was spiked into the nasopharyngeal swab specimens at the concentrations indicated in Table 3 and extracted with QIAamp Viral RNA Mini Kit before being analysed by PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit. The positive percentage agreement achieved 100% regardless of spike concentration. The positive percentage agreement of positive samples with the interference substance achieved 100% at 3X LOD while negative sample with the interference substance with percentage agreement achieved 100% as well with no false positive result interpretation.

Table 3. Results of Potential Interference Substance Study

Interference substance	Concentration of substance	Spike level	Positive percentage agreement (%)	Spike level	Negative percentage agreement (%)
Mucin	0.1 mg/mL	3X LoD	3/3 (100%)	Non-spiked	3/3 (100%)
Blood	1% (v/v)	3X LoD	3/3 (100%)	Non-spiked	3/3 (100%)
Nasal Spray	10% (v/v)	3X LoD	3/3 (100%)	Non-spiked	3/3 (100%)
Nasal Corticosteroid	10% (v/v)	3X LoD	3/3 (100%)	Non-spiked	3/3 (100%)
Nasal Wash	10% (v/v)	3X LoD	3/3 (100%)	Non-spiked	3/3 (100%)
Throat lozenges	1% (v/v)	3X LoD	3/3 (100%)	Non-spiked	3/3 (100%)
Antiviral	33 µg/mL	3X LoD	3/3 (100%)	Non-spiked	3/3 (100%)

Precision Study

A precision study is performed to evaluate the reproducibility (variability between different lots, days and operators) and repeatability (variability within a single run) of the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit. Percentage of agreement using different kit lots, on different days by different analysts as well as same user using one single lot were determined.

The result shows that the percentage agreement of positive samples achieves 100% using three different lots on all three days and between two operators with no false negative signal and within a single run by a single operator with no false negative signal. This demonstrates the performance of PHASIFY™ DeCOVID is reproducible between lots, days, operators and repeatable within lot and user.

Table 4. Reproducibility study result of PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit

		Day 1		Day 2		Day 3	
		Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
Lot	Condition	Percentage Agreement		Percentage Agreement		Percentage Agreement	
20F0073	Spiked sample 1	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Spiked sample 2	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Spiked sample 3	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
20F0074	Spiked sample 1	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Spiked sample 2	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Spiked sample 3	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
20F0091	Spiked sample 1	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Spiked sample 2	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Spiked sample 3	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

Table 5. Repeatability study result of PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit

Lot	Condition	Result Interpretation	Percentage Agreement
20F0074	Spiked sample 1	+	100% (10/10)
	Spiked sample 2	+	
	Spiked sample 3	+	
	Spiked sample 4	+	
	Spiked sample 5	+	
	Spiked sample 6	+	
	Spiked sample 7	+	
	Spiked sample 8	+	
	Spiked sample 9	+	
	Spiked sample 10	+	

Clinical Performance

Clinical performance of the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit was evaluated using 30 contrived positive and 30 negative nasopharyngeal swab specimens. Contrived positive nasopharyngeal swab specimens were prepared by spiking heat-inactivated SARS-CoV-2 virus from ATCC (Cat# VR-1986HK™) into SARS-CoV-2 negative nasopharyngeal swab specimen to a final concentration of 2X, 5X or 10X LOD. In total, 20 nasopharyngeal swab specimens spiked with 2X LoD, 6 nasopharyngeal swab specimens spiked with 5X LoD, 4 nasopharyngeal swab specimens spiked with 10X LoD and 30 SARS-CoV-2 negative nasopharyngeal swab specimens were extracted with QIAamp Viral RNA Mini Kit and analysed by PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR kit. The positive percentage agreement achieved 100% regardless of spike concentration. The negative sample percentage agreement was 100% for non-spiked SARS-CoV-2 negative specimens.

Table 6. Results of Clinical Evaluation Study

Spike level	Number of nasopharyngeal swab specimens	Agreement (%)
2X LoD	20	20/20 (100%)
5X LoD	6	6/6 (100%)
10X LoD	4	4/4 (100%)
Non-spiked	30	30/30 (100%)

Clinical Validation Study

A clinical validation study was performed to evaluate the performance of the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit. A total of sixty-four real patient respiratory tract samples consisting of 33 positive and 31 negative confirmed by EUA RT-PCR tests, were randomized and tested by the PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit. The results show 100% positive percentage agreement (33 out of 33) and 100% negative percentage agreement (31 out of 31) with the EUA RT-PCR test results.

Table 7. Results of Clinical Validation Study

		EUA RT-PCR test		
		Positive	Negative	Total
PHASIFY™ DeCOVID SARS-CoV-2 RT-qPCR Kit	Positive	**33 (100%)**	**0**	**33**
	Negative	**0**	**31 (100%)**	**31** (One of a sample that was originally recorded as positive but actually negative confirmed by Linea™ COVID-19 Assay Kit)
	Total	**33**	**31**	**64**

SYMBOLS

IVD	In vitro diagnostic *medical device*		*Manufacturer*
REF	*Catalogue number*		Caution
LOT	*Batch code*		Consult *instructions for use*
EC REP	Authorized representative in the European Community		Upper limit of temperature
Σ 100	Contains sufficient for *100* tests	CE	CE Marking
	Use-by date		

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PI-3010300UM1 | Rev A | For in vitro diagnostic use |

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