COVID-19 Nucleic Acid Fluorescence Kit (-15 to -35°C)

CATALOGUE NUMBER	KIT SIZE (TESTS)
PCRCOV1	50T

Intended Use:
The COVID-19 Nucleic Acid Fluorescence Kit is an assay for the qualitative detection of nucleic acid of SARS Cov-2 ORF1ab (open reading frame, ORF1ab) and N (Nucleoprotein, N) gene in human respiratory tract specimens.

Summary:
Coronaviruses are a large family of viruses that cause disease ranging from common cold symptoms to more severe pneumonia. They are enveloped, single strand RNA viruses. Coronaviruses are zoonotic, they can be transmitted from animals to humans. Existing examples include the Middle East Respiratory Virus (MER-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).
Reports of a novel coronavirus began in the Wuhan district of China in December 2019 and in January 2020 the World Health Organisation designated the new strain 2019-nCoV. Symptoms include high temperature, cough and breathing difficulties. In immunocompromised individuals, symptoms can be more severe leading to pneumonia, severe acute respiratory syndrome or death.

Test Principle:
The kit combines the principals of real-time PCR and nucleic acid detection by use of fluorescent probes to quantify the SARS Cov-2 ORF1ab and Nucleoprotein gene. The Enzyme Mix Reagent includes a uracil-DNA glycosylase (UDG) enzyme to cleave the uracil glycosidic bond of uracil-containing PCR fragments as an efficient mechanism to reduce false positive results caused by PCR product contamination.

Reagents:
The test kit comprises four tubes containing reagents of the following composition:

Tube 1 (950 µl) – ORF1ab/N PCR Buffer Mix – Primer and probes of Internal Control, primer and probes of SARS-Cov-2, dNTPs and $Mg2^{+}$ in buffer.
Tube 2 (65 µl) – Enzyme Mix – Revertase, Taq enzyme and UDG enzyme.
Tube 3 (250 µl) – Positive Control – Gene segment of SARS-CoV-2.
Tube 4 (250 µl) – Negative Control – Distilled water.

Instructions for Use sheet

Materials not provided: PCR analyser, viral RNA extraction kit, Class II biological safety cabinet, vortex, microfuge, nuclease free -centrifuge tubes and -PCR tubes/caps, micropipettes, sterile, nuclease free pipette tips and sterile, nuclease free distilled water.

Precautions:
For *in vitro* diagnostic use by trained professionals only.
Follow Good Laboratory Practice procedures where samples and kits are handled and treat the kit components and all samples as if potentially infectious. Follow local regulations for correct disposal of samples.
Wear protective clothing including laboratory coat, disposable gloves and safety glasses when conducting the test.
Do not use any component of the kit after the expiry date. Do not incorporate reagents from different kit batches or reagents from other commercially available kits.
The PCR amplification stage is exceedingly susceptible to cross-contamination. The work flow of all assay equipment and consumables must be from pre-amplification to post-amplification, never vice-versa.
To help prevent contamination, change gloves frequently and use different sterile, nuclease free pipette tips for each sample and reagent.
Never open the post-amplification tubes. Instead wrap and seal them up and discard the tubes along with unused reagent, assay consumables and assay waste according to local regulations.

Storage and Stability:
The kit must be stored frozen (-15 to -35°C) and protected from light. The kit is stable up to the expiry date printed on the outer label.

Sample Collection and Preparation:
The COVID-19 Nucleic Acid Fluorescence Kit can be performed using viral RNA extracted from upper and lower respiratory tract samples.
Upper respiratory tract samples should be collected via nasopharyngeal or pharyngeal wash or swabs by recommended technique.
Lower respiratory tract samples should be collected via bronchoalveolar lavage, tracheal extract or deep cough sputum by recommended technique.
Swab samples should be collected only using swabs with a synthetic tip and aluminium or plastic shaft. Do not use swabs with calcium alginate or cotton tips or with wooden shafts.

Ideally RNA extraction from the samples should be performed immediately. Alternatively, the samples may be stored up to 24 hours at 2 – 8°C or for longer at -70°C. Avoid repeat freeze-thawing.

Assay Procedure:
Viral RNA extraction reagents are not included in this kit. Extract RNA following the Instructions of Use for the viral RNA extraction kit of choice.

Viral RNA Detection
- Thaw the four COVID-19 Nucleic Acid Fluorescence Kit component tubes.
- When fully thawed, except the Enzyme Mix tube, mix the contents of the tubes by inversion ten times. Microfuge tubes by pulse action to force all volume to the base of the tube.
- Only microfuge the Enzyme Mix.
- Prepare a suitable volume of Working Mix by combining ORF1ab/N PCR Buffer Mix and Enzyme Mix in the following ratio according to the number of samples to be tested:

ORF1ab/N PCR Buffer Mix 18.75 µl
Enzyme Mix 1.25 µl (total volume = 20 µl / per test)

Important considerations:
Every PCR run must include one Positive Control and one Negative Control.
Do not vortex the Enzyme Mix or any tube containing Enzyme Mix.
All reaction mixes should be used immediately or as soon as possible after preparation.
Note: The Enzyme Mix is viscous, carry out all pipetting actions slowly to ensure full volume withdrawal and expulsion. Also do not lower pipette tip any further than necessary into the Enzyme Mix to prevent excess adherence of reagent on the outside of the tip.

- Label PCR tubes for Negative Control, Positive Control and each sample. Pipette 20µl of Working Mix into each tube.
- Add 5 µl Negative Control, Positive Control and sample into respective tubes then cap the tubes. (Prepare the Positive Control tube last to prevent contamination.)
- Microfuge PCR tubes by pulse action to force all volume to the base of the tube.
- Put the PCR tubes into a real-time PCR analyser.
- Programme the analyser according to the following cycling parameters:

Stage	Cycles	Temp (°C)	Time	Data
Reverse transcription	1	55	10 min	N/A
Initial denaturation	1	95	5 min	N/A
Cycle reaction	40	95	15 sec	N/A
		55	15 sec	N/A
		72	15 sec	FAM, HEX/VIC, ROX

- Initiate the PCR run immediately
- When the run is complete analyse the data, see below.

Settings
Baseline setting: Adaptive baseline.
Threshold setting: The Threshold line should be set just above the peak of the normal negative control amplification curve (irregular noise line).

Analysis of Results:
Assay Validation
- Check the results for the Controls first:

Control Result	FAM (Internal Control, β-actin)	HEX (ORF1ab gene)	ROX (N gene)
Negative Control	Ct < 40, with good amplification curve	No Ct or Ct = 0	No Ct or Ct = 0
Positive Control		Ct < 37	Ct < 37

- If Controls meet all the validation criteria above, proceed to sample analysis.

Sample Analysis

Sample Result	HEX (ORF1ab gene)	ROX (N gene)
Negative	No Ct or Ct = 0	No Ct or Ct = 0
Positive	Ct < 37	Ct < 37

If the result for HEX or ROX fall within the range 37 ≤ Ct < 40 the experiment must be repeated. If Ct <40 with a good amplification curve, the sample may be considered to be positive.
A sample is considered positive when ORF1ab and N of 2019-nCoV are both positive. Each sample defined positive using this kit should be repeated using a different detection method using re-extracted RNA.

Performance Characteristics:
Sensitivity limit: 1 x 10^3 copies/ml.
Coincidence rate of Positive reference: 100%.
Coincidence rate of Negative reference: 100%.
Precision: CV% ≤ 5%.

Limitations of the Test:
The COVID-19 Nucleic Acid Fluorescence Kit is only to be used by laboratory personnel trained in PCR techniques.
The results obtained with this assay should not be used as the sole criterion for diagnosis of COVID-19 infection, but be used in conjunction with other diagnostic procedures and clinical findings.
Results will only be accurate if samples are properly processed and correctly transported and stored. If the RNA is degraded the ability of this kit to detect COVID-19 will be compromised.

References:

1. World Health Organisation Statement regarding cluster of pneumonia cases in Wuhan, China: 9 January 2020.
2. Xintian Xu et al. Evolution of the coronavirus from the ongoing Wuhan outbrek and modelling of its spike protein for risk of human transmission. Published online 21 Jan 2020.
3. World Health Organisation. Coronavirus. www. who.int/health-topics /coronavirus.

Glossary of Symbols:

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		Do not reuse
	Keep away from sunlight		

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V1: rev Mar 2020