COVID-19 Lyophilised Nucleic Acid Kit

CATALOGUE NUMBER	KIT SIZE (TESTS)
PCRCOV1	50 Tests
PCRCOV2	500 Tests
PCRCOV3	1000 Tests

Intended Use:
The COVID-19 Lyophilised Nucleic Acid Kit is an in vitro assay for the qualitative detection of nucleic acid of SARS-CoV-2 ORF1ab (open reading frame, ORF 1ab) and N (Nucleoprotein, N) gene in human respiratory tract secretion samples.

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 China in December 2019 and in January 2020 the World Health Organisation designated the new strain 2019-nCoV (later SARS-CoV-2). 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 principles of real-time PCR and nucleic acid detection by use of fluorescent probes to quantify the SARS-CoV-2 ORF1ab and Nucleoprotein gene. The Lyophilised PCR Buffer Mix contains a uracil-DNA glycosylase 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. The probe is 5' labelled with fluorescent reporter and 3' labelled with fluorescent quencher. Before amplification the quencher absorbs the reporter and no fluorescent signal is emitted. During the polymerisation process Taq polymerase exerts 5'-3' exonuclease activity which degrades the probe and separates the reporter group and the quencher thereby enabling fluorescence emission and detection of an increase in amplification.

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

Tube	Contents	Volume	Quantity
1	Lyophilised PCR Buffer Mix	1000 µl	1 vial
2	Positive Control (lyophilised)	100 µl	1 vial
3	Negative Control	1500 µl	1 vial

Instructions For Use sheet

Materials not provided:, PCR analyser, viral RNA extraction kit, Class II biological safety cabinet, vortex, microfuge, nuclease-free -microcentrifuge tubes and -PCR tubes/caps, micropipettes, sterile nuclease-free pipette tips and sterile RNase-free and 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 device 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.
Compare the test reagents with the mode of action of the real-time PCR analyser. Compatible PCR analysers include Applied Biosystems 7500/Fast, Applied Biosystems StepOnePlus, Applied Biosystems QuantStudio 5/6/7, Roche Lightcycler 480II and BioRad CFX96 and the kit should run successfully on most other models.
Do not use the kit or any component after the expiry date. Do not substitute 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.
The Positive Control contains an RNA oligo of the native detection target so keep well away from the other reagents to prevent contamination which could lead to false positive results.
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 is to be stored at 2 - 8°C and protected against exposure to light. The kit is stable up to the expiry date printed on the outer label. The kit should be transported in a polystyrene lined box containing ice packs (2 – 28°C) and delivered within 14 days.

Sample Collection and Preparation:
The COVID-19 Lyophilised Nucleic Acid 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
- Remove freeze dried Lyophilised PCR Buffer Mix tube and reconstitute the Buffer Mix by adding 1000 µl nuclease-free water to form the Working Mix. Mix by repeatedly withdrawing and expelling the Mix using a pipette with tip then centrifuge briefly. When fully dissolved calculate the volume of Working Mix required for the first run which is the number of unknown samples + 2 multiplied by the volume of 20 µl/test of Working Mix. Immediately freeze the remainder of Working Mix in aliquots at -20°C or lower.
- Every PCR run must include one Positive Control and one Negative Control. Assign PCR tubes for these and for each sample being run.
- Pipette 20 µl Working Mix into each labelled tube.
- Bring RNA samples to room temperature and take out the Positive Control and Negative Control from the kit. Reconstitute the Positive Control tube with 100 µl nuclease-free water. Use 5 µl in the first run and freeze the remainder in aliquots at -20°C or lower.
- Add 5 µl Negative Control, 5 µl RNA sample, and 5 µl Positive Control (in that order) to assigned tubes using separate pipette tips. Cap the tubes.
- Briefly centrifuge the tubes to force all volume to the base of the tubes.
- Put the PCR tubes into a real-time PCR analyser.
- Programme the analyser according to the following cycling parameters:

Stage	Cycles	Temp (°C)	Time
Reverse transcription	1	50	30 min
Initial denaturation	1	95	5 min
Cycle reaction	45	95	10 sec
		55	40 sec

- 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	CY5 (Internal Control)	ROX (ORF1ab gene)	FAM (N gene)
Negative Control	No Ct or Ct = 0	No Ct or Ct = 0	No Ct or Ct = 0
Positive Control	Ct < 40, with good amplification curve	Ct < 35, with good amplification curve	Ct < 35, with good amplification curve

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

Sample Analysis

Sample Result	ROX (ORF1ab gene)	FAM (N gene)
Negative	No Ct or Ct = 0	No Ct or Ct = 0
Positive	Ct ≤ 35	Ct ≤ 35

If the result for FAM or ROX falls within the range 35 ≤ Ct < 40 the experiment must be repeated. If Ct is <35 with a good amplification curve, the sample may be considered to be positive.
A sample is considered positive when ORF1ab and/or N of 2019-nCoV are positive. Each sample defined positive using this kit should be repeated using a different detection method using re-extracted RNA.

Performance Characteristics:
Sensitivity limit: 500 copies/ml.
Coincidence rate of Positive reference: 98%.
Coincidence rate of Negative reference: 100%.
Precision: CV% ≤ 5%.

Limitations of the Test:
The COVID-19 Lyophilised Nucleic Acid 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 outbreak 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	2	Do not reuse
	Keep away from sunlight		

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