CoVirED

SARS-CoV-2 Extraction and Detection Kit

Instructions for life science research use only. Not tested for use in diagnostic procedures. For in vitro use only.

CoVirED - Corona Virus Extraction and Detection kit, RUO

Product is composed from:

A. **ViRNAEx RNA Extraction Kit (96 RNA extraction reactions)**
B. **SARS-CoV-2 RT-PCR Detection Kit (96 PCR reactions)**

Instructions For Use.

ViRNAEx RNA Extraction Kit (96 RNA extraction reactions)

Instructions For Use

SARS-CoV-2 RT-PCR Detection Kit

1. SARS-CoV-2 RT-PCR Detection Kit contents

Materials supplied with the kit (4 tubes):

- RT Mix Reverse Transcriptase (blue tube cap)
- Enzyme Mix, DNA Polymerase, buffer and dNTPs (green tube cap)
- Working Mix Primers and probes (amber tube cap)
- Positive control (synthetised DNA) (orange tube cap)

2. Additional equipment & reagents required (not provided in the kit)

- Real-Time PCR Systems have been tested (ABI7500, SLAN 96P and Bio-Rad CFX96 Real-Time PCR System)
- comaptible real -time PCR tubes, plates and accessories should be stored
- molecular biology grade water
- DNase and RNase-free pipette tips with aerosol barriers
- DNase and RNase-free tubes for preparing Reaction Mix
- Pipettes (adjustable)
- Vortex mixer
- Microcentrifuge

3. Shipment and storage

- Kit components must be shipped and stored frozen, (-20°C or below)
- Keep away from light to prevent photobleaching
- Kit is after opening stable up to the expiration date indicated on the packaging, if stored as recommended
- Avoid multiple freeze- thaw cycles

4.Description

The COVID-19 Diagnostic Kit is an *in vitro* real-time PCR-based test for the detection of two targets (*ORF1ab* and *N gene*) targets of SARS-COV-2 in a single tube assay. The test may be used in microbiology, molecular biology, molecular pathology laboratories or in epidemiological studies under research settings.
The positive control contains synthesized nucleic acid sequences for *ORF1ab* and *N gene* of SARS-COV-2 virus.

5. Sensitivity

The assay detects from 10 genome equivalent copies per PCR – reaction.

6. Material Safety Data (MSDS)

This product is not hazardous, toxic, or IATA-restricted. This product is not from human, animal or plant origin. According EU Directive 67/548/EC any products whit less than 1% of a hazardous component do not require a Material Safety Data Sheet (MSDS).

7. Warnings

The performance of the kit during RT-PCR may be affected by known PCR inhibitors (such as blood, excessive drugs presence– e.g.nasal sprays, eye drops and decongestants).

- The kit is designed for research use only
- The kit should be used by trained personnel with competency in real-time PCR
- Do not use any damaged kit components (check upon arrival) as they may not perform as expected
- Do not use the product beyond its expiry date
- Do not mix reagents from different batches
- The positive control provided in the kit must be used as a control in all PCR- experiments
- Fluorescently labelled probes are sensitive to photobleaching (please check the storage conditions)
- Personal protective equipment (PPE) should be worn during the work with kit components
- Appropriate DNase-free aerosol barrier pipette tips should be used
- Decontaminate working surfaces using wipes/sprays with 0.5% Sodium Hypochlorite solution or DNA AWAY™
- Unused reagents and waste should be removed in accordance with local regulations

8. Real time- PCR protocol

A. PCR Reaction Mix setup

- Kit components have to be defrosted, mixed by vertexing and briefly spun down before use
- All steps should be performed with minimal light exposure
- Precise number of reagents and test sample is of critical importance for accurate results

Prepare the Reaction Mix according to the table.

Product	**Tube cap colour**	**1 reaction volume (µl)**	**96 reactions volume (µl)**
Enzyme mix	**Green**	10.0	1000
RT mix	**Blue**	1.0	100
Working mix	**Amber**	2.0	200
Water (nuclease free)	**N/A**	2.0	200
Reaction mix (in total) *		**15**	**1500**
Positive Template Control (PTC)	**Orange**	5.0	**N/A**
Negative Template Control (NTC)	**N/A**	5.0	**N/A**
Tested sample (RNA) **	**N/A**	5.0*	**N/A**

* for any loss during pipetting it may be necessary to prepare an additional volume of reaction mix, a 5% excess is usually sufficient.

**5 µL of testing sample is recommended and should be used in most experiments. However, 2-8 µL of sample may be used, the volume of water must then be adjusted to ensure that the total reaction volume is 20 µLl.

Transfer 15 µL of the Reaction Mix into each of the wells of a PCR vessel, then add 5 µl of test sample, positive template control (PTC) or water (NTC) directly into each well as detailed in the table above. At least one PC and one NTC samples should be included per run.

The Positive Control should be used at 5 µL and the volume of additional water adjusted to ensure that the total reaction volume is 20 µL.

Please add the same total amount of reagents plus test sample to all PCR vessels. Seal the PCR plate using PCR caps and spin it down briefly. Every well should be sealed tightly to avoid evaporation.

B. Real-Time PCR Program

Regular PCR program (1) should be the first choice for standard use. If desirable, the Fast program (2) can be used, it significantly saves time, while the performance is not compromised.

1. **Regular PCR program**

STEP	**CYCLES**	**TEMPERATURE**	**TIME**	**DATA COLLECTION**
Reverse transcription	**1**	55°C	10 min	
Activation	**1**	95°C	1 min	
Amplification	**43**	95°C	10 sec	
		60°C	45 sec	End-point fluorescence collection
		69°C	10 sec	

2. **Fast PCR program**

STEP	**CYCLES**	**TEMPERATURE**	**TIME**	**DATA COLLECTION**
Reverse transcription	**1**	55°C	10 min	
Activation	**1**	95°C	1 min	
Amplification	**43**	95°C	10 sec	
		60°C	30 sec	End-point fluorescence collection

Data analysis

The data analysis is carried using ABI7500, Bio-Rad CFX96 or SLAN Real-Time PCR System software. Analysis is carried out in accordance with the software's instruction manual.

C. C.1 Setting the baseline, threshold and quantification cycle (Cq, Ct)

The baseline should be set to a range that eliminates the background fluorescence found in the early cycles of amplification, but which does not overlap the area where amplifications signals rise above the background. Baseline may be set automatically if it gives an acceptable value.

The cycle number at which a signal is detected above background fluorescence is termed the threshold cycle threshold (Ct) or quantification cycle (Cq). Select the threshold for Ct/Cq determination as close as possible to the base of the exponential phase.

Please note that ROX channel may have a slight background amplification when no target is present, you should set the baseline above this background amplification curve after inspecting NTC wells.

C.2 Sample analysis

1st step: Check the controls signal first, make sure that NTC gives no amplification signal and PTC gives positive signal in all channels. Then continue with the sample analysis.

2nd step: Check amplification in test samples. Amplification in FAM and ROX channels indicates the presence of ORF1ab gene and N gene of COVID-19, respectively.

Amplification in CY5 channel serves as an endogenous human gene control for monitoring that enough human genetic material was extracted.

The test is considered valid only if:

• NTC shows no amplification in all tested channels (Ct ≥40.0 or no Ct).
• **Positive controls show amplification in channels FAM, ROX and CY5 (Ct<39).**
• Samples should show amplification of CY5 channel Ct< 40), which indicates that some human genetic material is present, otherwise the sample may not have enough material and the result may be invalid.
• Samples are considered positive for SARS-COV-2 if there is amplification in both FAM and ROX channels with a Ct value ≤ 39.0.
• Samples are considered negative for SARS-COV-2 if there is absence of amplification in both FAM and ROX with a Ct value > 39.0 or no Ct.
• If one of channels (FAM or ROX) is positive, the sample is potentially positive and should be retested for confirmation.

9. Troubleshooting

Should you encounter problems please follow instructions bellow:

Absence of CY, ROX and/or FAM signal in PTC

- Check the instrument calibration records and confirm it is working.
- Check the storage temperature and whether the contents were exposed to prolonged direct sunlight.
- Verify that PCR cycling parameters correspond to those recommended above.

Absence of CY5 signal in Testing Samples

- The performance of the kit may be adversely affected by known PCR inhibitors
- If the samples show strong amplification in FAM and/or ROX channels, it is possible that competition from an excess of COVID-19 RNA may have repressed Internal control amplification Result valid

10. Glossary

PCR Polymerase Chain Reaction
COVID-19 Infection caused by SARS-COV-2
NTC No Template Control
IC Internal Control
PC Positive Control

11. Customer contact information

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