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SARS-CoV-2 Surface kit

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MBK0092- 96 tests

INTENDED USE

SARS-CoV-2 Surface kit allows the qualitative detection of SARS-CoV-2-RNA in environment samples (surface and fomites).
The kit is intended to be used by analytical laboratories for environmental surface samples as part of quality control/quality assurance testing. The kit is not intended for clinical diagnostics and shall be intended for research use only although it can be used to monitor the presence of SARS-CoV-2 on surfaces and fomites.

INTRODUCTION

Coronaviruses are a large family of viruses that are common in people and many different species of animals including camels, cattle, cats, and bats.
In December 2019, a cluster of patients with a novel coronavirus was identified in Wuhan, China. The virus named SARS-CoV-2 can cause the disease named coronavirus disease 2019 (COVID-2019) [WHO, 2 March 2020]. The virus seems to be transmitted mainly via small respiratory droplets through sneezing, coughing, or through people interaction in close proximity. These droplets can then be inhaled, or they can land on surfaces that others may come into contact with, who can then get infected when they touch their nose, mouth or eyes. The SARS-CoV-2 can survive on different surfaces from several hours (copper, cardboard) up to a few days (plastic and stainless steel) (https://www.ecdc.europa.eu/en/covid-19/questions-answers). Therefore, environmental testing for the detection of SARS-CoV-2 could play a crucial role in the Covid-19 outbreak investigation and allow the businesses, individuals, state agencies to perform adequate decisions for the public health.
The test is based on the WHO guideline [Berlin protocol, 2nd revision Corman et al. 2020].

PRINCIPLE OF THE ASSAY

The **SARS-CoV-2 Surface kit** is a One-step real-time reverse transcription (RT-PCR) multiplex assay based on fluorescent-labelled probes used to confirm the presence of SARS-CoV-2-RNA by amplification of **RdRp**. The kit also detects an Internal Process Control, that it can be added directly to the sample material to guarantee reproducible and reliable results.
The kit provides all the reagents required for the analysis. PCR positive and PCR negative controls are also included.

KIT CONTENTS

Reagent	No vial x Volume
Mix 1	1 X 550 µL
Mix 2	1 X 70 µL
Mix Primer/Probe	1 X 1030 µL
ROX 20uM	2 X 50 µL
PCR Negative Control	1 X 100 µL
PCR Positive Control	1 X 50 µL

REQUIRED MATERIALS NOT SUPPLIED

- Internal Process Control
- Disposable powder-free gloves
- RNA isolation kit
- Pipettes
- Sterile pipette tips with aerosol-preventive filters
- Vortex mixer
- Bench Microcentrifuge
- Real-time PCR instrument

- Consumables for real-time PCR instruments
- 1.5mL tubes
- Laboratory freezers -30°C to -10°C/-70°C
- Cold block or ice

ASSAY LIMITATIONS

- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper sample collection, transfer, storage and processing may cause erroneous test results
- The kit uses purified RNA as sample for the analysis. The quality of the RNA recovered from biological samples is essential for the quality of results generated with this kit.
- False negative results may arise from:
  - Improper sample collection
  - Degradation of viral RNA during shipping/storage
  - Using poor extraction method
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instruction for use
- False positive results may arise from:
  - Cross contamination during specimen collection handling or preparation
  - RNA contamination during product handling

SHIPPING AND STORAGE CONDITIONS

Shipping on dry ice. Upon arrival, store at -20°C. If stored at the recommended temperature all reagents are stable until the expiration date.

WARNING AND PRECAUTIONS

Before using the kit read the Product Information carefully and completely.
The operator should always pay attention to:

- Set up pre- and post-PCR areas. Do not share instruments or consumables (pipettes, tips, tubes etc) between those areas;
- Store positive material separately from all other reagents and, if possible, add it to the reaction mix in a separated space;
- Do not use any reagent after the expiration date indicated on the label;
- Wear powder-free gloves during all procedures;
- Thaw all kit components and protect from light before starting the assay. After thawing, mix the components and centrifuge briefly;
- Minimize sample handling;
- Change gloves frequently;
- Wash the bench surfaces with 5% sodium hypochlorite;
- Use sterile disposable laboratory materials and do not re-use the tubes and tips;
- Store the reagents at the recommended temperature;
- Dispose waste in compliance with the local regulations;
- Positive results are indicative of the presence of SARS-CoV-2 RNA
- The quality of the sample preparation may influence the quality of the RT-PCR test.

INSTRUCTIONS FOR USE

1. SAMPLE PREPARATION

1.1 Sample preparation

Sampling procedures should be done according to "Surface sampling of coronavirus disease (COVID-19): A practical "how to" protocol for health care and public health professionals" version 1.1, 18 February 2020, WHO.
The samples should be extracted according to the corresponding requirements and procedures of viral RNA extraction kits [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance].
The kit has been tested on RNA samples extracted using various methods. For additional information please contact Diatheva.
The extracted RNA can be directly tested. Alternatively, please store the RNA sample at below -70°C, avoiding repeated freeze-thaw.

1.2 Internal Process Control

The volume of Internal Process Control depends on the final elution volume. **For this application, the volume of Internal Process Control must be 1/100 of the elution volume (i.e., add 0.5 µL Internal Process Control for 50µL elution volume).**

3. PROGRAM SETUP

Program the PCR instrument before preparing the reaction mix.
The kit has been optimized to be used with QuantStudio 3-5, ABI 7500, CFX96 and Rotor gene Q 2-plex e 5-plex thermal cyclers. For compatibility with other instruments please contact Diatheva.

- Program the real-time PCR instrument with the following thermal profile:

Step	Temperature and Times	Cycles
cDNA Synthesis	48°C 30 min	1 X
Initial denaturation	95°C 10 min	1 X
Denaturation	95°C 15 sec	45 X
Annealing-extension*	60°C 30 sec	

*Fluorescence is detected during **annealing-extension** step on:

- green channel (FAM dye) for the target **RdRp**;
- yellow channel (VIC/Cal Fluor orange 560 dye) for the **Internal Process Control**;

Select **ROX** as passive reference dye for instruments that require it (es. Applied Biosystems).

The final reaction volume is **20µL**.

4. PCR MIX PREPARATION

All detection experiments should include a PCR Negative Control (NTC-No Template Control), containing all the components of the reaction except for the template. This enables detection of potential contamination.

- Thaw the components protect from light. Vortex Mix 1, Mix Primer/Probe for 15 sec and centrifuge briefly. Vortex Mix 2 for 2 sec and centrifuge briefly.
- In one sterile 1.5 ml tube prepare the amplification reaction mix (Master mix) needed for each sample to be tested plus one Negative control and one Positive control following the pipette scheme below:

Reagent	1 reaction*
Mix 1	5 µL
Mix 2	0.625 µL
Mix Primer/Probe	9.375 µL
Total volume	15 µL

*For the analysis of more than one sample, simply multiply the volumes of Mix 1, Mix 2 and Mix Primer/Probe for the number of samples to be tested considering the NTC and Positive Control.

- Vortex for 15" the vial containing the prepared Master mix and centrifuge briefly
- Aliquot **15µL of Master mix** in the PCR tubes or in the plate prepared for the experiment
- Add **5µL of PCR Negative Control** in the corresponding tube
- In a separate area, add **5µL of RNA samples to be tested**, into the corresponding PCR tubes or wells containing amplification mix
- Add **5µL of Positive Control** into the corresponding PCR tubes or wells containing amplification mix
- Seal hermetically the PCR tubes or plate and load into the real-time PCR instrument, following the manufacturer's instructions

Note: for PCR instruments with 96 wells block check that the reagents are at the bottom of each well, if not, briefly centrifuge at 800 x g for 1 minute.

4.1 PCR MIX PREPARATION ONLY for instruments that require HIGH ROX concentrations (Step-One and Step One Plus)

NOTE: Follow the protocol described below ONLY if you are using an instrument that requires HIGH ROX (Step-One and Step One PLUS)

All detection experiments should include a PCR Negative Control (NTC-No Template Control), containing all the components of the reaction except for the template. This enables detection of potential contamination.

- Thaw the components protect from light. Vortex Mix 1, Mix Primer/Probe for 15 sec and centrifuge briefly. Vortex Mix 2 for 2 sec and centrifuge briefly.
- In one sterile 1.5 ml tube prepare the amplification reaction mix (Master mix) needed for each sample to be tested plus one Negative control and one Positive control following the pipette scheme below:

Reagent	1 reaction*
Mix 1	5 µL
Mix 2	0.625 µL
Mix Primer/Probe	9.375 µL
ROX 20uM	0.45 µL
Total volume	15 µL**

*For the analysis of more than one sample, simply multiply the volumes of Mix 1, Mix 2 and Mix Primer/Probe for the number of samples to be tested considering the NTC and Positive Control.
**Please consider to aliquot 15 µL. The addition of ROX must not be considered

- Vortex for 15" the vial containing the prepared Master mix and centrifuge briefly
- Aliquot **15µL of Master mix** in the PCR tubes or in the plate prepared for the experiment
- Add **5µL of PCR Negative Control** in the corresponding tube
- In a separate area, add **5µL of RNA samples to be tested**, into the corresponding PCR tubes or wells containing amplification mix
- Add **5µL of Positive Control** into the corresponding PCR tubes or wells containing amplification mix
- Seal hermetically the PCR tubes or plate and load into the real-time PCR instrument, following the manufacturer's instructions

Note: for PCR instruments with 96 wells block check that the reagents are at the bottom of each well, if not, briefly centrifuge at 800 x g for 1 minute.

5. ANALYSIS OF RESULTS

The analysis of the results should be done with the PCR instrument program, please refer to the manual for detailed information. Set the baseline and threshold values. Some software perform the data analysis automatically in this case it is advisable to check these settings. For a manual data analysis, analyze the PCR file for the three fluorophores separately. For a correct definition of the threshold it is necessary to select a value distinction from the background after the linear phase growth.

5.1 Run validity

The use of positive and negative controls in each run validates the reaction by checking the presence of signal for SARS-CoV-2 in the positive control well and the absence of signal in the negative control well. Before interpreting the sample results you need to verify the PCR run.
If results differ from those indicated in the tables below the PCR run is not valid and must be repeated.

QuantStudio, ABI 7500 and CFX96

Control ID	Detection Channel	
	FAM (Green channel) Ct	**VIC/Cal Fluor orange 560 (Yellow channel) Ct**
PCR Positive Control	Positive	Positive
PCR Negative Control (NTC)	No amplification signal	No amplification signal*

*When white plastic (plates or strip) for Real-Time PCR is used it could be observed the presence of faint signal that must not be considered for the interpretation

Rotor-Gene Q

Control ID	Detection Channel	
	FAM (Green channel) Ct	**VIC/Cal Fluor orange 560 (Yellow channel) Ct**
PCR Positive Control	Positive	Positive
PCR Negative Control (NTC)	No amplification signal	No amplification signal

5.2 Interpretation of results

Ct value		Interpretation of Result
RdRp	**Internal Process Control**	
Ct≥10	Not significant*	SARS-CoV-2 RNA Positive
Negative	Ct≤40	SARS-CoV-2 RNA Negative
Negative	Ct>40	Invalid result (re-test)

*A sample is considered positive if the Internal Process Control is Positive. However, sometimes, this target cannot be amply due to preferential amplification of RdRp.

6. INSTRUMENT COMPATIBILITY

The kit is for use with:

- CFX96 Biorad
- ABI 7500
- QuantStudio 5
- QuantStudio 3

- Rotor gene Q 2- plex e 5-plex
- For use with other thermal cyclers please contact Diatheva

7. REFERENCES

WHO Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases, 2 March 2020

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M.L. and Mulders, D.G., 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 25(3).