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COVID-19 PCR DIATHEVA Detection kit

Version: **02-April 2020**

REF **D-MBK0091** Σ 96 IVD CE

INTENDED USE

COVID-19 PCR DIATHEVA Detection kit allows the qualitative detection of SARS-CoV-2-RNA in upper and lower respiratory samples 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 observations.
Testing with the kit is intended for use by laboratory trained personnel in the technique of real-time PCR and in vitro diagnostic procedures.

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].
PCR testing of asymptomatic or mildly symptomatic contacts can be considered in the assessment of individuals who had contacted with a COVID-19 case.
The test is based on the WHO guideline [Berlin protocol, 2nd revision Corman et al. 2020].

PRINCIPLE OF THE ASSAY

The **COVID-19 PCR DIATHEVA Detection kit** is a One-step real-time reverse transcription (RT-PCR) multiplex assay based on fluorescent-labelled probe used to confirm the presence of SARS-CoV-2-RNA by amplification of **RdRp** and **E gene**. The assay includes also **RNase P** target as internal positive control (IC) to evaluate extraction of RNA and the presence of PCR inhibitors. 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
PCR Negative Control	1 X 100 µL
PCR Positive Control	1 X 50 µL

REQUIRED MATERIALS NOT SUPPLIED

- 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 at room temperature has no detrimental effect on the performance of this kit. 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 qPCR test.

INSTRUCTIONS FOR USE

1. SAMPLE PREPARATION

1.1 Sample preparation

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 and refer to Appendix 1.
The extracted RNA can be directly tested. Alternatively, please store the RNA sample at below -70°C, avoiding repeated freeze-thaw.

3. PROGRAM SETUP

Program the PCR instrument before preparing the reaction mix.
The kit has been optimized to be used with QuantStudio 5, ABI 7500, CFX96 and Rotor gene Q 5-plex thermal cyclers. For the 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	50 X**
Annealing-extension*	58°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 **E gene**;
- red channel (quasar 670/Cy5) for **RNase P**.

** Please consider 45 cycles for the use with Rotor gene Q 5-plex

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

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

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 protects 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 **15 µL of Master mix** in the PCR tubes or in the plate prepared for the experiment
- Add **5 µL of PCR Negative Control** in the corresponding tube
- In a separate area, add **5 µL of RNA samples to be tested**, into the corresponding PCR tubes or wells containing amplification mix
- Add **5 µL 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.

Threshold values setting

Target	CFX96	ABI7500	QS5	Rotor gene Q 5-plex*
RdRp	80	0.1	0.1	0.06
E	50	0.05	0.05	0.08
RNaseP	30	0.02	0.02	0.08

*after threshold values setting please carry out the analysis by setting at 20 the number of cycles to ignore at the beginning of the cycling.

5.1 Run validity

The use of positive and negative controls in each run validate 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. Evaluate the controls according to the table below:

Control ID	Detection Channel		
	FAM (Green channel)	VIC/Cal Fluor orange 560 (Yellow channel)	Quasar670/Cy5 (Red channel)
PCR Positive Control	Positive	Positive	Positive
PCR Negative Control (NTC)	No amplification signal	No amplification signal	No amplification signal*

*the presence of aspecific amplification signal (Ct valueε45) can appear but it can be considered as trascurable

If the run is valid, continue with the interpretation of the sample results according to table below. Alternatively, if the run is not valid please consider to repeat before to proceed.

5.2 Interpretation of results

Ct value			Interpretation of Result
RdRp	**E**	**RNaseP (IC)**	
≤45	≤45	≤40*	SARS-CoV-2 RNA Positive
≤45	N/A	≤40*	
N/A	≤45	≤40	SARS-CoV-2 RNA Negative**
N/A	N/A	≤40	SARS-CoV-2 RNA Negative
N/A	N/A	N/A	Invalid result (re-test)

*A sample is considered positive if the Ct value obtained is less than 40. However, sometimes, this target cannot be amply due to preferential amplification of RdRp or E.
****** Repeat the test. Possible infection with others Coronavirus

6. INSTRUMENT COMPATIBILITY

The kit is for use with:

- CFX96 Biorad
- ABI 7500
- QuantStudio 5
- Rotor gene Q 5-plex
- For use with other thermal cyclers please contact Diatheva

7. PERFORMANCES CHARACTERISTICS

ANALYTICAL SPECIFICITY
The specificity of primers and probes can be ensured through the use of specific sequences evaluated by *in silico* analysis and amplification under stringent conditions.

DIAGNOSTIC SENSITIVITY
A significant number of samples positive for SARS-CoV-2 were tested with the COVID-19 DIATHEVA Detection kit and simultaneously with another device. The results obtained made it possible to calculate a diagnostic sensitivity of 100%.

DIAGNOSTIC SPECIFICITY
A significant number of negative samples for SARS-CoV-2 negativity were tested with the COVID-19 DIATHEVA Detection kit and simultaneously with another device. The results obtained made it possible to calculate a diagnostic specificity of 100%.

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).