Datasheet

SARS-CoV-2 (2019-nCoV) virus detection system (N and ORF1 genes assays)
Cat: K168 (2x1,25 ml = 100 Tests)

1. Description

SARS-CoV-2 (2019-nCoV) virus detection system is a kit for efficient *in vitro* detection of coronavirus SARS-CoV-2 (or 2019-nCoV) based on one-tube cDNA synthesis and Real-Time PCR. Kit includes two primers and probe set for detection and confirmation steps.

Real-time RT-PCR technology include the reverse transcription reaction (RT) stage to convert RNA to complementary DNA (cDNA) and the polymerase chain reaction (PCR) stage to amplify and detect a target sequence using specific primers and fluorescent probes.

2. Components

Component	200 reactions (100 tests)
2× qPCR Master Mix	2 × 1.25 ml
BioMaster-mix	1 × 200 µl
Primer-probe set ORF1b	1 ×75 µl
Primer-probe set N	1 ×150 µl
Positive control (PTC) ORF1b	1 × 100 µl
Positive control (PTC) N	1 × 100 µl
Non-Template Control (NTC)	1 × 200 µl

3. Instruments:

The kit can be used on the following PCR instruments:

- LightCycler® 96 (Roche)
- LightCycler® 480 (Roche)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

4. Experimental design:

1) Initially, all samples are tested using a system of primers and probe N with the setting of the corresponding positive and negative controls.
2) Next, positive samples must be re-confirmed using a primer system and an ORF1b probe with the appropriate positive and negative controls.

Select the FAM channel for the detection of the amplification signal of the N and ORF1b genes.

5. Procedure

5.1 Sample preparation:

Extraction of the encapsidated RNA is mandatory before use in real time RT-PCR. The quality of the extracted RNA has a profound impact on the performance of the entire test system. It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology.

.. a good decision ..

5.2 Protocol

Screening (detection).

All reagents and samples should be completely thawed, mixed (by pipetting or gentle shaking) and discarded droplets by centrifugation.

Prepare reagent premixes with a set of primers and probe N, based on the following formula: (2+x+1) reaction mixtures, where 2 show reactions with PTC and NTC, x is the number of samples and +1 additional volume to compensate for the possible pipetting error.

Confirmation.

At the confirmation stage, prepare reagent premixes with a set of primers and an ORF1b probe, from the following formula: (2+ y +1) reaction mixtures, where 2 show reactions with PTC and NTC, y - the number of samples positive for the N gene assay, and +1 additional volume to compensate for the possible pipetting error

Component calculation:

Component	**1 reaction**	**7 reactions**
2× qPCR Master Mix	12,5	125
BioMaster-mix	1,0	10
Primer-probe set N/ORF1b	1,5	15
Total	**15,0**	**150**

5.3 Reaction setup

Take a 96-well plate, add using an automatic pipette 15.0 µl of the prepared premix for detection or confirmation stage. Add 10.0 µl of sample solution (solution after RNA isolation) and controls (PTC and NTC) to the appropriate cells containing premixes with different primers.

Make sure positive and negative controls have been added.

Gently mix sample and control solutions with premixes by pipetting. Remember to change the tip of the automatic pipette!

Close the 96-well reaction plate with appropriate covers or optical adhesive film. If you use reaction tubes, make sure they are suitable for use in real-time amplifiers.

Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at a speed of approximately 1000 x g (~ 3000 rpm).

For basic information on setting up and programming various real-time PCR tools, please refer to the user manual of the corresponding tool.

5.4 Suggested thermal cycling conditions

Step	Temperature	Time	Cycles
Reverse Transcription	45 °C	30 min	1
Initial activation	95 °C	5 min	1
Denaturation	95 °C	10 sec	45
Annealing and extension*	56 °C	30 sec	

* detection in FAM channel

6. Data analysis and interpretation:

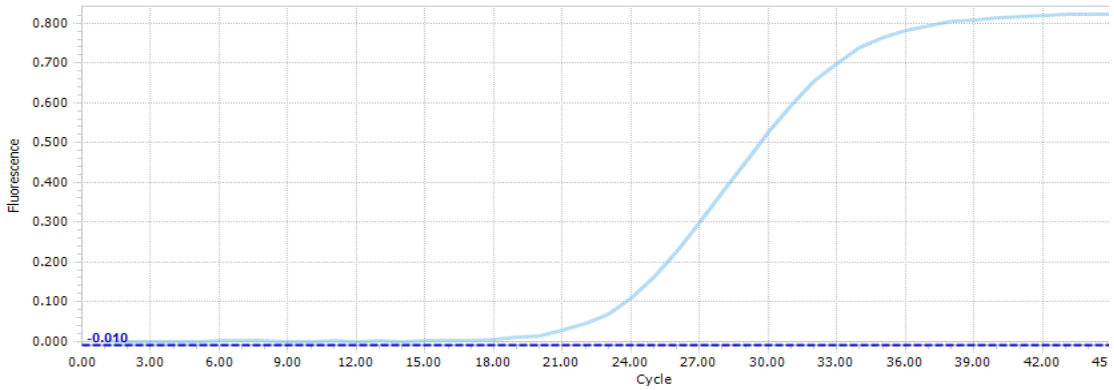
Screening

At the screening stage, the researcher identifies samples in which SARS-CoV-2 RNA is present.

PCR was successful and the data are reliable subject to the following criteria:

- in the negative control, the signal in the FAM channel is absent or Ct > 37 cycles;
- in the positive control in the FAM channel, the signal corresponds Cq 22-26.

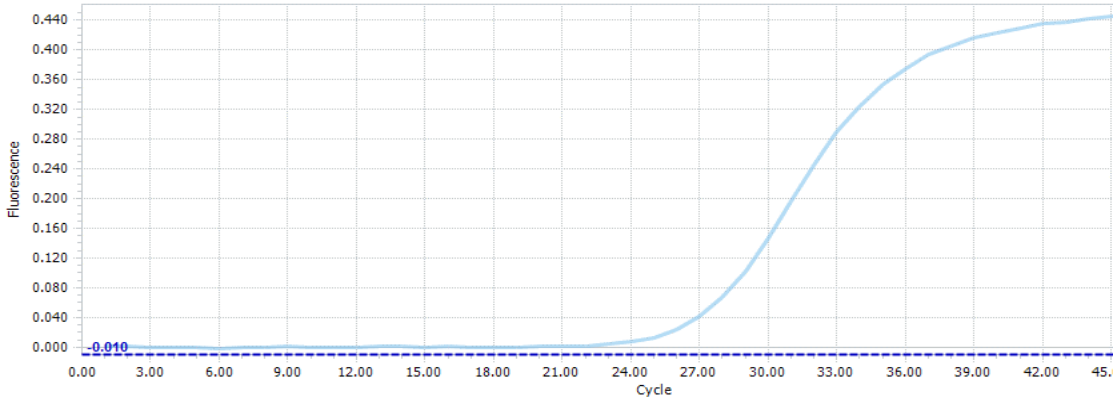
Test on detection of HKU-N RNA positive control:



Samples in which a signal with Ct values less than 37 is detected in the FAM channel are subjected to further analysis in the Confirmation stage.
Samples where the signal is negative (Ct > 37 cycles) in the FAM channel do not contain RNA of SARS-CoV-2 virus.

Confirmation
At the Confirmation step, the researcher confirms that SARS-CoV-2 virus RNA is present in the sample.
PCR was successful and the data are reliable subject to the following criteria:

- in the negative control, the signal in the FAM channel is absent or Ct > 38 cycles
- in the positive control in the FAM channel, the signal corresponds Cq 25-29.
Test on detection of HKU-ORF1b RNA positive control:



Samples in which a signal with Cq values less than 38 is observed in the FAM channel are positive for the presence of RNA of SARS-CoV-2 virus.
Samples in which the signal is negative in the FAM channel are undefined and require confirmation by the reference system or send to the reference laboratory.

7. Storage and transportation
Storage terms: in a place protected from light at +4 ° C – 1 week; at -20 ° C - 1 year; not more than 30 thawing-freezing cycles.
Transportation: at 0 - +4 ° C.
Limitation: Research Use Only!

Ordering Information: Cat.-No: K168; SARS-CoV-2 (2019-nCoV) virus detection system; 100 Tests

Reference:
Chu D. K. W. et al. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. Clinical Chemistry, hvaa029