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# User Manual Novel coronavirus (2019-nCoV-2) Food Check kit

RT-qPCR

For professional use only

## USER MANUAL

**REF** CV19F

DNA Diagnostic A/S

[www.dna-diagnostic.com](http://www.dna-diagnostic.com)

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## 1. PURPOSE OF THE TEST

The novel coronavirus SARS-CoV-2 (2019-nCoV-2) is known to cause COVID-19, which is a respiratory illness spreading from person to person via direct contact with or exhaled respiratory droplets generated when an infected person coughs or sneezes. Although the SARS-CoV-2 cannot grow on food due to a virus requiring a living host like a person or an animal to multiply. Considering that SARS CoV-2 can remain viable on surfaces for quite a period of time it may survive in foods and on surfaces for hours to days. It is essential to develop innovative diagnostics for SARS-CoV-2 not only for infected people but also for food and surrounding environments. The food industry is required to maintain trust and consumer confidence in the safety of food. Novel coronavirus (2019-nCoV-2) Food Check kits from DNA Diagnostic will enable food producers to determine, and monitor the presence of SARS-CoV-2 in food production and environment. Using a reliable RNA extraction from 25 grams from food matrix categories (as presented in the Table 1) followed by an accurate, sensitive and highly specific RT-qPCR reaction, the result can be acquired in 3 hours. The kit is intended for professional use only - intended users are trained laboratory personnel instructed in the procedure. The procedure must be performed in a biosafety cabinet.

**Table 1**

<b>Matrix category</b>	<b>Matrix type</b>	<b>Matrix items (examples)</b>
<b>Raw meat and ready-to-cook meat products</b> (except poultry)  Sample size: <b>25g</b>	Fresh meats (unprocessed)	Meat cuts (beef cube), minced meat (ground pork)
<b>Raw poultry and ready-to-cook poultry products</b>  Sample size: <b>25g</b>	Fresh meats (unprocessed)	Meat cuts (chicken fillet)
<b>Ready-to-eat and ready-to-reheat meat products, poultry products and fishery products</b>  Sample size: <b>25g</b>	Cooked meats	Chicken nugget

## 2. KIT COMPONENTS AND STORAGE

The kit contains the following materials.

- Novel coronavirus SARS-CoV-2 (2019-nCoV-2) Detection kit

**Kit Contents for 36 tests**

Tube No.	Contents	Main Ingredients	Quantity
1	nCoV Reaction Mix	Primers, Probes, Mg <sup>2+</sup> , dNTPs	1200 mL/tube
2	nCoV Enzyme Mix	Taq DNA Polymerase, Reverse Transcriptase	40 µL/tube
3	nCoV Positive Control	Plasmid DNA	100 µL/tube

### Storage and Stability

The kit requires shipment on frozen ice packs. All contents of the kit should be stored immediately upon receipt at  $-20\pm 5^{\circ}\text{C}$  and protected from light.

The shelf-life of the kit is six months. Vial-opening does not affect the shelf-life of the kit. The recommend maximum freeze-thaw cycle is five cycles.

### 3. REQUIRED EQUIPMENT AND REAGENTS BUT NOT SUPPLIED

- Compatible PCR instruments: Stratagene Mx3000P™, ABI7500
- Biosafety cabinet
- Vortexer
- Mini centrifuge with rotor for centrifuge tubes.
- Heating block that can be adjusted between 56-80°C
- Personal Protective Equipment (PPE): Disposable powder-free gloves, goggles/face shield, respirators (NIOSH-certified N95 is recommended)
- Nuclease-free centrifuge tubes
- Nuclease-free PCR tubes and caps
- Adjustable pipettes and sterile, nuclease-free pipet tips with filter
- Biohazard waste container
- Tube racks
- Sterile, nuclease-free water
- Blender bag with full-page filter (porosity of the filter at 280 microns is recommend).

## 4. PRECAUTIONS AND HANDLING REQUIREMENTS

### Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation
- Please check the compatible real-time PCR instruments prior to use.
- The quality and concentration of the RNA sample greatly affects the results of this test. To minimize the risk of degradation by ribonucleases, we strongly recommend purification of total RNA immediately after taking
- Always store aqueous RNA solutions at -80°C. Even an overnight storage at -20°C may result in RNA degradation. Avoid repeated freeze-thawing
- When working with RNA always use gloves, as hands are a major source of ribonuclease contamination.
- The integrity and purity of RNA is important for the efficiency of the cDNA synthesis and thus detection of translocations. The quality of RNA can be checked by OD 260/280 nm measurements, agarose gel electrophoresis, or using RIN number from the Agilent Bioanalyzer.
- The kit is intended for professional use only - intended users are trained laboratory personnel instructed in the procedure. The procedure must be performed in a biosafety cabinet.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

### Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Wear appropriate personal protective equipment (PPE) when working with specimens.
- Specimen processing should be performed in a certified Class II biosafety cabinet following biosafety level 2 or higher guidelines. Follow relevant biosafety regulations and use appropriate personal protective equipment. Consult with current national and WHO guidelines on biosafety for COVID-19 testing [1,2].
- The products can be discarded as normal laboratory waste
- Check the safety data sheet for the product on our website for further information or ask for a copy of it.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.

- DO NOT pipette by mouth.
- Avoid aerosols.

### **Decontamination and Disposal**

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes and nuclease-free filtered pipette tips when handling samples and reagents to prevent exogenous RNA contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly.
- DO NOT open the post- amplification PCR tubes.
- All disposable materials are for one-time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

### **Cleaning**

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

## 5. PROTOCOL

### Sample preparation

1. Sample should be cut into small pieces before it is inserted on one side of the filter in the culture bag.
2. Add PBS pH7.2-7.4 to the culture bag with sample matrix in the ratio 1:1 (e.g. 25 g of sample in 25 mL of PBS).
3. Mix manually for 5-10 seconds. Do not blend (stomach)/homogenize the sample and the PBS.
4. For each sample, transfer 1 mL liquid from the other side of filter in culture bag to a clean 1.5 mL tube.
5. Centrifuge at 1000xg for 3 minutes.
6. Transfer 300 µL supernatant of each sample into new 1.5 mL tube.
7. Perform viral RNA extraction.

### Viral RNA extraction:

1. Please follow the instruction manual which is provided with the DNA Diagnostic Virus/Cell RNA Kit (Cat. No. CV19E) for RNA extraction.

### Viral RNA Detection:

1. Every PCR run must contain one PC (Positive control) and one NTC (No template control).
2. Take the nCoV Reaction Mix, nCoV Enzyme Mix and nCoV Positive Control out of the kit from the freezer.
3. Thaw nCoV Reaction Mix and nCoV Positive Control at room temperature. When the reagents completely thawed, invert each tube for 10 times and centrifuge briefly to collect the contents at the bottom of the tube.
4. Centrifuge the nCoV Enzyme Mix prior to use.
5. Take out the sample RNA and nuclease-free water for NTC.
6. Prepare sufficient nCoV Master Mix containing nCoV Enzyme Mix and nCoV Reaction Mix in separate sterile centrifuge tube as indicate in the table below

Content	Volume per test
nCoV Reaction Mix	29.9
nCoV Enzyme Mix	1.1
<b>Total</b>	<b>31.0</b>

#### Note:

- Do not vortex enzyme mix or any mixture with enzyme mix.
- The prepared mixtures should be used immediately, avoid prolonged storage.



- Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.
  - Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.
7. According to the ratio in Table above. Mix nCoV Master Mix thoroughly by gently pipetting up and down more than 10 times, and then centrifuge briefly.
  8. Prepare PCR tubes for NTC, sample, PC. Then dispense 31  $\mu$ L nCoV Master Mix into correspondence PCR tube.
  9. Add 9  $\mu$ L NTC, 9  $\mu$ L sample RNA or 9  $\mu$ L nCoV Positive Control (nCoV-PC) to the correspondence PCR tubes. Cap the PCR tubes.  
Note: Suggest following below adding order: NTC  $\rightarrow$  samples  $\rightarrow$  PC, and use filtered tips for all pipetting steps to avoid cross-contamination.
  10. Briefly centrifuge the PCR strips to collect all liquid at the bottom of each PCR tube.
  11. Setup the PCR Protocol using the cycling parameters in Table below.

Stage	Cycles	Temperature	Time	Data collection
1	1	55°C	15 min	
		95°C	5 min	
2	45	95°C	25 s	FAM, HEX and ROX
		55°C	35 s	
		72°C	20 s	
3	1	40°C	30 s	

12. Start the PCR run immediately.
13. When the PCR run is finished, analyse the data according to the “Results Interpretation” procedures.

### Results Interpretation:

14. Before data analysis, the following items should be checked:
  - a. Analyse single fluorescent signal each time and select the PC amplification curve to adjust the threshold value. Choose the reaction wells of PC, IAC, NTC and sample simultaneously for analysis.
  - b. For NTC: the FAM and ROX Ct values should be > 45 (negative) while the HEX Ct values should be  $\leq$  34. If not, the data is INVALID. The sample should be retested.
  - c. For NTC: the IAC HEX Ct values should be  $\leq$  34. If not, the data is INVALID. The sample should be retested.
  - d. For Positive Control: the FAM and ROX Ct value should be in the range from 31-34. If not, the data are INVALID. The sample should be retested.

## Analyse the result for each sample:

15. Check the Internal Amplification control HEX signal for each sample:
  - a. For NTC: The HEX Ct value should be  $\leq 34$ .
  - b. For NTC: If the HEX Ct value is  $> 34$ , this indicates the reaction may contain of PCR inhibitors, the sample should be retested with diluted or re-extracted RNA.  
*Note: the HEX Ct can be  $> 34$  or missing if a Ct for FAM and/or ROX is low.*
16. Check ORF1ab FAM and N ROX signals for each sample:
  - a. If any of Ct value (either ORF1ab FAM or N gene ROX Ct value) is  $\leq 37$ , the sample is determined as SARS-CoV-2 positive.
  - b. If both Ct values (ORF1ab FAM and N gene ROX Ct value) are  $> 40$ , the sample is determined as SARS-CoV-2 negative or below the LOD.
  - c. If any of Ct value (either ORF1ab FAM or N ROX Ct value) is  $> 37$  and  $\leq 40$ , retest the sample. If any of Ct value (either ORF1ab FAM or N gene ROX Ct value) is still  $\leq 40$  in the retest result, the sample is determined as SARS-CoV-2 positive. If both Ct values (ORF1ab FAM and N gene ROX Ct value) are  $> 40$  in the retest result, the sample is determined as SARS-CoV-2 negative or below the LOD.

## 6. INSTRUMENT SETUP

- Setup the reaction volume as 40  $\mu\text{L}$ .
- For Stratagene Mx3000P™, please set up the Filter Set Gain Settings of FAM, HEX and ROX as 2. If there's low net fluorescence signal (dR) but high background signal (R), please reduce the signal gain setting of instrument properly.
- For ABI 7500, the kit is only available on Standard instead of Fast. Please set up as follows: Reporter Dye: FAM, HEX and ROX; Quencher Dye: NONE; Passive Reference: NONE.
- For LightCycler480 II instrument, it's necessary to conduct Color Compensation prior to the first use according to Color Compensation instructions. Please contact DNA Diagnostic Technical Support or Account Manager to get the DNA Diagnostic novel coronavirus (2019-nCoV) Detection Kit – Calibration Reagent (for color compensation) and instructions. Refer to the real-time PCR instrument operator's manual for detailed instructions.





We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

## References

[1] WHO: Laboratory biosafety guidance related to coronavirus disease (COVID-19) (Link: [https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-\(covid-19\)](https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19)))

[2] WHO: Laboratory Biosafety manual, third edition (link: <https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1>)

## Symbols

<b>REF</b> <i>Catalogue Number</i>	 LOT Lot number	<b>CONT</b> <i>Contents</i>
 <i>Storage temperature</i>	 Expiry Date	 Manufacturer

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