

2019-nCoV TaqMan RT-PCR Kit

Product# TM67100, TM67120

Product Insert

Intended Use

Norgen's 2019-nCoV TaqMan RT-PCR Kit is designed for the detection of SARS-CoV-2 specific RNA in a real-time RT-PCR based on the use of TaqMan® technology. Norgen's 2019-nCoV TaqMan RT-PCR Kit provides SARS-CoV-2 detection based on the assays and protocols developed by the Centers for Disease Control and Prevention (CDC). This kit is designed for research use only and not for use in diagnostic procedures.

Background Information

In December 2019, an outbreak of respiratory illness started in Wuhan City, Hubei Province, China and has now spread throughout the world to many different countries. This respiratory disease was caused by a novel coronavirus and was initially termed "2019 novel coronavirus" or "2019-nCoV", however in February of 2020 the World Health Organization (WHO) announced that the official name of the disease is COVID-19. The official name of the coronavirus causing COVID-19 is SARS-CoV-2.

SARS-CoV-2 is a new strain of coronavirus infecting humans that had not been previously detected before the outbreak in China in December 2019. While SARS-CoV-2 is new, many coronaviruses have been known to infect animals and humans for some time. Coronaviruses are known to commonly infect camels, cattle, cats, and bats. In humans, Coronavirus infections can cause various illnesses from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS).

Human infection is more severe when the coronavirus has originated in animals and spread to humans, as is the case with MERS and SARS. SARS-CoV-2 is a betacoronavirus, similar to MERS and SARS, both of which have their origins in bats. The animal source of SARS-CoV-2 has not yet been identified, however Chinese officials have linked many of the early cases to a large seafood and live animal market, suggesting that the initial transmission was the result of animal-to-person spread. However, many of the later detected cases did not report any exposure to animal markets, indicating that the virus is now spreading via human-to-human contact.

Symptoms of infection with SARS-CoV-2 can range from milder symptoms such as a runny nose, sore throat, cough, and fever to more severe symptoms including pneumonia or breathing difficulties. In some cases infection with SARS-CoV-2 has resulted in death. As with other respiratory illnesses, older people and individuals with pre-existing medical conditions (such as diabetes or heart disease) are more vulnerable to becoming severely ill with the virus.

Product Description

Norgen's 2019-nCoV TaqMan RT-PCR Kit is designed for the detection of SARS-CoV-2 specific RNA in a real-time RT-PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures. The detection of SARS-CoV-2 specific RNA is based on TaqMan one-step RT-PCR providing a simple, reliable and rapid result for the detection of SARS-CoV-2 infection. Norgen's 2019-nCoV TaqMan RT-PCR Kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The 2019-nCoV TaqMan RT-PCR Kit comprises Master Mix for the target and PCR control detection, 3 target Primer & Probe Mixes, as well as a positive control and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.

Norgen's 2019-nCoV TaqMan RT-PCR Kit provides SARS-CoV-2 detection based on the assays and protocols developed by the CDC. The Primer & Probe Mixes contain all 3 CDC developed assays in individual tubes. All assays are premixed to the working concentrations recommended by the CDC. The Positive Control contains two nCoV nucleocapsid target gene RNA (N1 and N2) and RNase P (internal control).

Norgen's 2019-nCoV TaqMan RT-PCR Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q, BioRad CFX96 Touch™ Real-Time PCR Detection System, ABI 7500

Kit Components

Component	Product # TM67100 (50 reactions)	Product # TM67120 (500 reactions)
2019-nCoV_N1 Probe/Primer Mix	80 µL	850 µL
2019-nCoV_N2 Probe/Primer Mix	80 µL	850 µL
RNAse P Probe/Primer Mix	80 µL	850 µL
2019-nCoV RT-PCR Positive Control	50 µL	500 µL
2X One-Step RT-PCR Master Mix	2 x 1 mL	20 x 1 mL
Nuclease-Free Water (Negative control)	1.25 mL	10 x 1.25 mL
Product Insert	1	1

Storage Conditions and Product Stability

- All kit components should be stored at -20°C upon arrival
- Repeated thawing and freezing (> 2 x) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.
- All reagents can be stored for 6 months at -20°C without showing any reduction in performance.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument with FAM filter channel
- RNA Purification Kit
 - The kit is compatible with all RNA purification kits that yield high quality, inhibitor-free RNA
 - **Recommended Purification Kit:** Norgen's Total RNA Purification Kit (Cat. 17200, 37500)
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- PCR reaction preparation station

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's 2019-nCoV TaqMan RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Disclaimer

Norgen Biotek is not an endorsed supplier of the CDC coronavirus RT-PCR panel or positive controls. Norgen is offering these products based on the sequences that were published by the CDC, and these are being offered to our customers for research use only, not for *in vitro* diagnostic use

Warnings and Precautions

- Norgen's 2019-nCoV TaqMan RT-PCR Kit is intended for research purposes only. It is not intended for diagnostic use.
- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Dispose of unused kit reagents and specimens according to local, provincial or federal regulations.
- Do not substitute or mix reagents from different kit lots or from other manufacturers. Do not use components of the kit that have been stored for more than 6 months.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the SARS-CoV-2 genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.
- Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

Instructions for Use

A. Sample Preparation

Purified RNA is the starting material for Norgen's 2019-nCoV TaqMan RT-PCR Kit. The quality of the RNA template will have a major impact on the performance of the SARS-CoV-2 detection test. The user must ensure that the method used for RNA purification is compatible with TaqMan One-Step RT-PCR. We recommend the use of Norgen's **Total RNA Purification Kit (Cat. 17200, 37500)**.

B. TaqMan RT-PCR Assay Preparation

Notes:

- Before use, suitable amounts of all TaqMan RT-PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.

- Work quickly on ice.
- The amount of 2X One-Step RT-PCR Master Mix provided is enough for up to 50 RT-PCR reactions per each target for Cat# TM67100, or up to 500 RT-PCR reactions per target for Cat# TM67120
- For every TaqMan One-step RT-PCR run, one reaction containing 2019-nCoV Positive Control and one reaction as no template control must be included for proper interpretation of results. The recommended minimum number of RNA samples tested per TaqMan One-step RT-PCR run is 10. See the Example of Sample and Control Set-up in Table 1 below

Table 1. Example of Sample and Control Set-up

Target		1	2	3	4	5	6	7	8	9	10	11	12
2019-nCoV_N1	A	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	COVID-PC
2019-nCoV_N2	B	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	COVID-PC
RNAse P	C	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	COVID-PC
2019-nCoV_N1	D	NTC	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	COVID-PC
2019-nCoV_N2	E	NTC	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	COVID-PC
RNAse P	F	NTC	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	COVID-PC

- To avoid any contamination while preparing the TaqMan One-step RT-PCR assay, follow the order outlined in Tables 2, 3 and 4 below to prepare the Negative Control, Detection Assay and Positive Control:
 1. Prepare the RT-PCR Negative Control (Table 2)
 2. Prepare the RT-PCR 2019-nCoV Assay (Table 3)
 3. Prepare the RT-PCR Positive Control (Table 4)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (i.e: 1) Nuclease-free water; 2) Primer & Probe Mix; 3) Mastermix; and 4) the Sample RNA or Positive Control).

1. For each TaqMan One-step RT-PCR set, prepare **three** no template control PCR reactions as shown in Table 2 below:

Table 2. TaqMan One-step RT-PCR Negative Control Preparation

Reagent	Vol. of Reagent Added per Reaction
Nuclease-Free Water	8.5 µL
2X One-Step RT-PCR Master Mix	10 µL
2019-nCoV Primer & Probe Mix*	1.5 µL
Total Volume	20 µL

* Three different reactions will be prepared using each of the 3 provided Primer & Probe target mixes: 2019-nCoV_N1 Probe/Primer Mix, 2019-nCoV_N2 Probe/Primer Mix, RNAse P Probe/Primer Mix

2. Prepare the **three** RT-PCR reactions for sample detection as shown in Table 3 below.

Table 3. TaqMan One-step RT-PCR 2019-nCoV Assay Preparation

Reagent	Vol. of Reagent Added per Reaction
Nuclease-Free Water	3.5 µL
2X One-Step RT-PCR Master Mix	10 µL
2019-nCoV Primer & Probe Mix*	1.5 µL
Sample RNA+	5 µL
Total Volume	20 µL

* Three different reactions will be prepared for each sample using each of the 3 provided Primer & Probe target mixes: 2019-nCoV_N1 Probe/Primer Mix, 2019-nCoV_N2, RNase P Probe/Primer Mix

+ The recommended amount of sample RNA to be used is 5 µL. However, a volume between 1 and 5 µL of sample RNA may be used as template. Adjust the final volume of the RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

3. For each RT-PCR set, prepare **three** positive control RT-PCR as shown in Table 4 below:

Table 4. TaqMan One-Step RT-PCR Positive Control Preparation

Reagent	Vol. of Reagent Added per Reaction
2X One-Step RT-PCR Master Mix	10 µL
2019-nCoV Primer & Probe Mix*	1.5 µL
2019-nCoV Positive Control (PosC)+	5 µL
Nuclease-Free Water	3.5 µL
Total Volume	20 µL

* Three different reactions will be prepared for each sample using each of the 3 provided Primer & Probe target mixes: 2019-nCoV_N1 Probe/Primer Mix, 2019-nCoV_N2 Probe/Primer Mix, RNase P Probe/Primer Mix

+ The positive control contains the CDC 2019-nCoV markers (N1 & N2) and RNase P gene which are compatible with the CDC 2019-nCoV specific primer/probe sets

C. 2019-nCoV TaqMan One-Step RT-PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run one step RT-PCR.

Table 5. 2019-nCoV TaqMan One-Step RT-PCR Program

One Step RT-PCR Cycle	Step	Temperature	Duration
<i>Cycle 1</i>	Step 1	50°C	30 min
<i>Cycle 2</i>	Step 1	95°C	3 min
<i>Cycle 3 (45x)</i>	Step 1	95°C	3 sec
	Step 2	55°C	30 sec

D. 2019-nCoV TaqMan One-Step RT-PCR Assay Interpretation

- The Negative Control (NTP – No Template Control) reaction(s) must be negative and not exhibit fluorescence growth curves that cross the threshold line. If there is any amplification with the NTP the run is not valid and no interpretation of 2019-nCoV detection can be made. The assay must be repeated.
- The Positive Control (PosC) reaction(s) should produce a positive result with an expected Ct value (< 40.00 Ct) for each target. If the positive control does not provide a positive result the run is not valid and no interpretation of 2019-nCoV detection can be made. The assay must be repeated.
- If the NTC and PosC are exhibiting the correct results, the results of the detection assays can be interpreted as outlined in Table 6 below

Table 6. Interpretation of Assay Results

2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct Values	Result
+	+	±	< 40.00 Ct	2019-nCoV Detected
If only one (1) of two targets is positive		±	< 40.00 Ct	Inconclusive Result
-	-	+	< 40.00 Ct	2019-nCoV Not Detected
-	-	-	N/A	Invalid Result

Related Products	Product #
2019-nCoV Primer & Probe Mix	TM67101, TM67130
2019-nCoV RT-PCR Positive Control	PC67102, PC67120
Swab Collection and Total Nucleic Acid Preservation System	68800
Saliva RNA Collection and Preservation Devices	RU53800
Total RNA Purification Kit	17200, 37500, 17250
2X One-Step RT-PCR Master Mix	28113, 28114, 28115

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

Norgen's 2019-nCoV TaqMan RT-PCR Kit is designed for the detection of SARS-CoV-2 specific RNA in a real-time RT-PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen's 2019-nCoV TaqMan RT-PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR.

Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

The presence of PCR inhibitors may cause false negative or invalid results.

Potential mutations within the target regions of the SARS-CoV-2 genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

The respective user is liable for any and all damages resulting from application of Norgen's 2019-nCoV TaqMan RT-PCR Kit for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

TaqMan is a registered trademark of Roche Molecular Systems, Inc

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