



COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Product# TM67240

Product Insert

Intended Use

Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) is a multiplexed assay designed for the qualitative detection of SARS-CoV-2 specific RNA using TaqMan® technology (FAM and HEX/VIC) based on the Charité/Berlin protocol. The assay can be used on RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples collected from individuals with clinical signs/symptoms related to SARS-CoV-2 infection. 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) includes 2X One-Step RT-PCR Master Mix and 2 primer/probe mixes, a positive control and a negative control (nuclease-free water). The first primer/probe mix is used for first line screening and contains the E gene/RP that targets the SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs Envelope gene (E gene - FAM) in addition to the human RNase P transcript (RP - HEX/VIC) as an internal control target to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The second Primer/Probe Mix is only required as a confirmatory/discriminatory step with samples showing positive amplification of the E gene. This second Primer/Probe Mix is for the RdRP gene and detects two RNA-dependent RNA Polymerase (RdRP) targets where the first RdRP target is SARS-CoV-2 specific (FAM) while the second RdRP target is to detect SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs (HEX). The provided E gene/RdRP/RP Positive Control contains an *in vitro* RNA transcript for the three SARS-related target genes: E gene, RdRP gene as well as the human RP gene (internal control).

Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 # TM67240 (40 reactions)
E gene/RP Primer & Probe Mix	80 µL
RdRP gene Primer & Probe Mix *	80 µL
E/RdRP/RP Positive Control †	50 µL
2X One-Step RT-PCR Master Mix	1 mL
Nuclease-Free Water (Negative control)	1.25 mL
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* Confirmatory/Discriminatory assay

† Contains an *in vitro* RNA transcript for the three SARS-related target genes: E gene, RdRP gene as well as the human RP (internal control).

Storage Conditions and Product Stability

- All kit components should be stored at -20°C upon arrival
- Repeated thawing and freezing (> 3x) 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 and HEX/VIC 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 Saliva/Swab RNA Purification Kit (Cat. 69100)
- 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) is tested against predetermined specifications to ensure consistent product quality.

Disclaimer

Norgen Biotek is offering the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) based on the sequences that were published by the Charité/Berlin protocol (<https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045>). These products are for research use only, not for *in vitro* diagnostic use.

Warnings and Precautions

- Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes). 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 **Saliva/Swab RNA Purification Kit (Cat. 69100)**.

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 40 RT-PCR reactions per each target.

- For every TaqMan One-Step RT-PCR run, one reaction containing E gene/RdRp/RP Positive Control and one reaction as a no template control (NTC) must be included for proper interpretation of results. A minimum number of 10 samples are recommended to be tested per run per assay. Table 1 and Table 2 below show an example for the samples and the controls set-up for each assay.
- For SARS-CoV-2 detection, E gene/RP Primer & Probe Mix is required for initial detection. RdRP gene Primer and Probe Mix is then used in a separated RT-PCR reaction as a confirmatory/discriminatory assay to validate positive samples detected by the E gene.
- The kit is used in 2 stages; the first is to perform line screening to test if the sample is positive for SARS-CoV-2, SARS-CoV or bat-SARS-related CoVs. Positive samples are then used in a second PCR (Confirmatory/discriminatory PCR) to determine if the sample is SARS-CoV-2 positive or SARS-CoV or bat-SARS-related CoVs positive.

Table 1. Samples and Controls Set-up for the E gene/RP Assay

Assay	1	2	3	4	5	6	7	8	9	10	11	12
E gene/RP	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Positive Control

**Table 2. Samples and Controls Set-up for the RdRP gene Assay*
(Confirmatory/Discriminatory Step)**

Assay	1	2	3	4	5	6	7	8	9	10	11	12
RdRP genes	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Positive Control

* Only samples showing positive E gene amplification should be confirmed by the RdRP gene assay in a separated RT-PCR reaction.

- To avoid any contamination while preparing the TaqMan One-step RT-PCR assay, follow the order outlined in Tables 3, 4 and 5 below to prepare the NTC, Detection Assays and E gene/RdRp/RP Positive control:
 1. Prepare the RT-PCR NTC (Table 3)
 2. Prepare the RT-PCR E gene/RP Assay or RdRP gene Assay (Table 4)
 3. Prepare the RT-PCR E gene/RdRp/RP Positive Control (Table 5)
- 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 no template control PCR reactions as shown in Table 3 below:

Table 3. TaqMan One-Step RT-PCR NTC Preparation

Reagent	Volume of Reagent Added per Reaction
Nuclease-Free Water	8.5 µL
2X One-Step RT-PCR Master Mix	10 µL
E gene/RP Primer & Probe Mix *	1.5 µL
Total Volume	20 µL

* The RdRP gene Primer & Probe Mix can be used instead to validate positive samples detected by the E gene

2. Prepare the RT-PCR reactions for sample detection as shown in Table 4 below.

Table 4. TaqMan One-Step RT-PCR Target Assays Preparation

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

* The RdRP gene Primer & Probe Mix can be used instead to validate positive samples detected by the E gene.

+ The recommended amount of sample RNA to be used is 5 µL. However, 1 µL - 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 in case the volume of the sample RNA used is different from the volume shown in Table 4.

3. For each RT-PCR set, prepare positive control RT-PCR as shown in Table 5 below:

Table 5. TaqMan One-Step RT-PCR E gene/RdRP/RP Positive Control Preparation

Reagent	Vol. of Reagent Added per Reaction
2X One-Step RT-PCR Master Mix	10 µL
E gene/RP Primer & Probe Mix *	1.5 µL
E gene/RdRP/RP Positive Control +	5 µL
Nuclease-Free Water	3.5 µL
Total Volume	20 µL

* The RdRP gene Primer & Probe Mix can be used instead to validate positive samples detected by the E gene.

+ The positive control contains the SARS-CoV-2 E gene, RdRP gene and RNase P RNA fragments.

C. COVID-19 TaqMan One-Step RT-PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 6 below.
2. Run one step RT-PCR.

Table 6. COVID-19 TaqMan One-Step RT-PCR Program

One Step RT-PCR Cycle	Step	Temperature	Duration
<i>Cycle 1</i>	Step 1	50°C	20 min
<i>Cycle 2</i>	Step 1	95°C	3 min
<i>Cycle 3 (45x)</i>	Step 1	95°C	15 sec
	Step 2	58°C	30 sec

D. COVID-19 TaqMan One-Step RT-PCR Assay Interpretation

- The Negative Control (NTC – 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 NTC the run is not valid and no interpretation of SARS-CoV-2 detection can be made. The assay must be repeated.
- The **E gene/RdRP/RP Positive Control** 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 SARS-CoV-2 detection can be made. The assay must be repeated.
- **Only samples showing a positive signal for E gene should be re-tested with RdRP gene for confirmation.**
- Table 7 below shows the targets and specificity of the primer/probes used in this assay
- If the NTC and E gene/RdRP/RP Positive Control are exhibiting the correct results, the results of the detection assays can be interpreted as outlined in Tables 8 and 9 below

Table 7. Target and Specificity of Primer/Probes

Assay	Target	Specificity
Initial Screening	E gene (FAM)	SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs
	RP gene (HEX)	Human transcriptome
Confirmatory / Discriminatory	RdRP Confirmatory (FAM)	SARS-CoV-2
	RdRP Discriminatory (HEX)	SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs

Table 8. Interpretation of Assay Results with E gene/RP Primer & Probe Mix

E gene (FAM)	RP (HEX)	Result
+	+	Positive
+	-	Positive
-	+	Negative
-	-	PCR inhibited

Table 9. Interpretation of Assay Results with RdRP gene Primer & Probe Mix

RdRP Confirmatory Detection (FAM)	RdRP Discriminatory Detection (HEX)	Result
+	+	SARS-CoV-2 specific detection
-	+	SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs Positive
-	-	Negative
+	-	Invalid PCR

Related Products	Product #
Total Nucleic Acid Preservation Tubes (50)	69200
Saliva RNA Collection and Preservation Devices	RU53800
Saliva/Swab RNA Purification Kit	69100
Saliva/Swab RNA Purification 96-Well Kit	69300
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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) is a multiplexed assay designed for the qualitative detection of SARS-CoV-2 specific RNA using TaqMan® technology (FAM and HEX/VIC) based on the Charité/Berlin protocol. The assay can be used on RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples collected from individuals with clinical signs/symptoms related to SARS-CoV-2 infection. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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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