

Data sheet

MaxSen One-Step RT-qPCR Kit

[Coronavirus Detection (Strain 2019-nCoV)]

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Cat. No: COVID-19-1Sen (100 rxns)

Introduction

A novel coronavirus, currently termed 2019-nCoV, was announced as the etiological agent of cases of ongoing pneumonia outbreak in Wuhan City. This kit describes the use of real time RT-PCR for the *in vitro* detection of 2019-nCoV in respiratory specimens (sputum; *nasopharyngeal, oropharyngeal aspirates, washes or swabs; tracheal aspirates*).

MaxSen One-Step RT-qPCR Kit allows efficient cDNA synthesis and Real-Time PCR in a single tube. The kit includes a qPCR master mix supplied in a 2X to perform real-time PCR. The 2X qPCR Master Mix contains all the reagent (except PCR primers, probe and template) needed for running PCR reactions. In addition, a separate RT mix that comprises a balanced mixture of both RTase (Reverse Transcriptase) and RNase Inhibitor is also provided.

Also, the kit contains one set of primers and fluorescent probes to differentiate between 2019-nCoV and SARS-CoV (or bat SARS related CoV). The probes are readout in different channels. Coronavirus 2019-nCoV RNA targets are amplified and detected in the FAM channel, Coronavirus SARS-CoV (or bat SARS related CoV) RNA targets are amplified and detected in the HEX, VIC or JOE channel (depending on the equipment used).

The assay includes positive control (PTC) and negative control (NTC). The positive control is supplied to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm absence of contamination, a negative control reaction should be included every time the kit is used.

Each kit contains:

- ✓ 2X qPCR Master Mix (1 vial)
- ✓ RT mix (1 vial)
- ✓ PCR Primer/ Probe set (1 vial) *
- ✓ Rehydration Buffer (1 vial)
- ✓ COVID-19 Positive control (PTC) (1 vial)
- ✓ Non-Template Control (NTC) (1 vial)

Storage

MaxSen One-Step RT-qPCR Kit is shipped on dry/blue ice. The Kit should be stored at -20°C upon receipt. Avoid repeated freezing and thawing. Maintain cold when thawed.

*PCR Primers/ Probe Set must be rehydrated **in the dark**. Mix gently and aliquot in different tubes. Store aliquots at -20°C.

Features

- ✓ **Inclusivity:** SARS like coronavirus and specific detection of 2019-nCoV
- ✓ **Dual Color Multiplex Assay Format**

Product use limitation

In response to the new coronavirus (2019-nCoV) emergency, this kit is intended to facilitate and support research. This kit is a method of assistant diagnosis for research use only and cannot be used as a basis for confirming or excluding cases.

Research Use Only (RUO). This kit has not been tested and validated by any public health agency.

PCR Primer/ Probe set is referenced in publicly available 2019-nCoV real-time PCR protocols (information can be found on the [WHO website](http://www.who.int)).

BASIC PROTOCOL

1. Thaw kit components on ice. Mix each solution well.

The following protocol is recommended for a 20 µl reaction volume:

2. Set up the following reaction mixture.

Component	Volume reaction *
2X qPCR Master Mix	10 µL
RT mix	1 µL
PCR Primer/ Probe set	4 µL

*Multiply all numbers according to experimental requirements

3. After mixing reagents above, distribute 15µL into the number of wells required for your testing. (include 1 well for the NTC and 1 well for the PTC)

4. Add 5 µL of RNA extracted from each sample, NTC and PTC in different wells and close them with the provided caps.

The quality of the test depends on the quality of the RNA sample. Unsuitable collection, storage and/or transport of specimens may give false negative results.

5. Program the appropriate PCR cycling protocol on your real-time PCR instrument

Suggested thermal cycling conditions

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	10 min	1
Initial activation	95°C	3 min	1
Denaturation	95°C	15 sec	45
Annealing and extension	58°C*	30 sec	

*Acquisition must be performed at the end of this stage

6. Select the fluorescent channel (FAM/ HEX) of instrument for testing.

2019-nCoV = FAM (465-510)

SARS-CoV (or bat SARS related CoV) = VIC / HEX / JOE (533-580)

Analysis of results

Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data.

Controls

- ✓ **Non-Template Control (NTC)** should be negative and not exhibited Ct value in FAM channel and HEX channel. If NTC reaction is positive, sample contamination has been occurred.
- ✓ **Positive Template Control (PTC)** should be positive and exhibited an expected Ct value for each channel included in the kit. If these results are not obtained, repeat the assay implementing corrective actions for failed reactions.

Samples

When all controls exhibit the expected performance then the samples could be positive, negative or suspicious

- ✓ **Positive:** If $Ct \leq 35$ in both FAM and HEX channel.
- ✓ **Negative:** If there is no Ct value in any of the FAM channel and HEX channel.
- ✓ **Suspicious samples:**
 - If there is no Ct value in any of the FAM and HEX channel, it is recommended to re-extract RNA. If the result is the same, the sample can be reported as negative.
 - Samples with Ct value greater than 35, it is recommended to re-extract RNA for RT-PCR. If the result is still less than 40, the sample can be reported as positive, otherwise it is negative.

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. Please refer to www.novateinbio.com for the Material Safety Data Sheet of the product.