This protocol was kindly developed and provided by

Department of Medical Sciences, Ministry of Public Health, Thailand.

This document contains RT-PCR protocol for the detection of 2019-nCoV.

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Diagnostic detection of Novel coronavirus 2019 by Real time RT-PCR

Materials & Methods

1.1 Macherey-Nagel Nucleospin RNA virus (Cat. No 740956)
1.2 Invitrogen superscript™ III Platinum One-Step Quantitative (Cat No. 11732-020 or 11732-088)

Primer	Sequence (5′ → 3′)	Working conc.
WH-NIC N-F	CGTTTGGTGGACCCTCAGAT	40 µM
WH-NIC N-R	CCCCACTGCGTTCTCCATT	40 µM
WH-NIC N-P	FAM-CAACTGGCAGTAACCA-BQH1	10 µM

Real-time RT-PCR Set-up Procedure

Place your samples on ice. Follow the procedure below to prepare the RT-PCR Master Mix.

a. Prepare the Master Mix as shown in the table below.
b. Pipette 20 µl of the Master Mix into each required reaction tubes/plate.
c. Add 5 µl isolated RNA or 5 µl the controls (Positive Control or Blank Control).
d. Make sure that every run including at least one Positive Control and one Blank Control.
e. Cap or seal the reaction tubes/plate and centrifuge using an appropriate centrifuge for 30 seconds at approximately 2,000 rpm.
f. Ensure that all liquid is at the bottom of the tubes/plate.
g. Perform the following protocol in the instrument.

Reagents	microliter/r	Thermal cycler condition

	eaction			
Rnase free H_2O	5.5	**Round**	**Temp.**	**Time**
2x PCR Master Mix	12.5	1X	50^0C	30 min.
Forward primer (40 µM)	0.5	1X	95^0C	2 min.
Reverse primer (40 µM)	0.5	45X	95^0C	15sec.
Probe (10 µM)	0.5		55^0C**	30sec.**
Superscript –Tag Mix	0.5			
Total	20.0	** Fluorescence data (FAM) collect		
Add RNA	5			

Data Analysis and Interpretation

- Threshold setting : Above the maximum level of Blank Control
- Quality control : Prior to evaluating the specimen results, the Positive Control and Blank Control should be interpreted. Negative control should be undetected. Positive control should be detected with Ct ≤ 38
- The Positive Control and Blank Control should be included per PCR run.
- If the Positive Control and Blank Control do not meet the criteria, the entire run is invalid and results should not be reported. Repeat the entire process (specimen and control preparation, amplification and detection).
- Viral transport media or negative specimen can be used as a negative control.