



DIAKEY REALcheck COVID-19(nCOV) Detection Kit

Code No: MF01, 100 reactions / in vitro diagnostics for swab

1. INTENDED USE

Diakey 2019_nCoV Detection Kit is for detection of new Coronavirus(COVID-19) and determining the presence of 2019_nCoV (COVID-19) infection in human clinical specimens such as a nasal or tracheal swab samples.

2. PRINCIPLE OF THE ASSAY

DIAKEY 2019_nCoV Detection Kit comprises all reagents and control DNA for detection of viral RNA of 2019-nCoV(COVID19) and the qualitative testing of DIAKEY 2019_nCoV Detection Kit is based on Multiplex Real-Time RT-PCR system: In one reaction setting the target genes for 2019_nCoV(COVID-19) and internal control (IC) RNA or DNA derived from human specimens are reverse transcribed (Reverse Transcription, RT) and amplified in parallel by respective primer pairs in the subsequent Polymerase Chain Reaction (PCR). Amplified target gene fragments are detected via fluorescently labeled probes during the PCR reaction in real time (Real-Time RT-PCR) and probes specific for detection of amplified 2019_nCoV (COVID-19) and IC-RNA target genes are respectively labeled with fluorescent dyes FAM, Texas Red and HEX. Their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler instrument. By means of both individual analyses in one reaction vessel per sample and the Negative Control (NC) and Positive Control (PC) per run the 2019_nCoV (COVID-19) status of a sample can be evaluated in the end. This way, results can be achieved within few hours after sample receipt.

This Kit was developed for use by trained laboratory personnel following standardized procedures. This direction for use must be followed strictly.

3. REAGENTS AND MATERIALS

DIAKEY 2019_nCoV Detection Kit contains the following reagents:

Reagent	Quantity in Kit With 100 reactions	Storage conditions
2X OneStep qRT-PCR Master Mix (For Probe)	1mL x 1EA	-20°C to -15°C
Primer & Probe Mixture (PM) (RdRp/N/RP)	0.2mL x 1EA	-20°C to -15°C
Positive Control DNA(PC) (RdRp/N)	0.8mL x 1EA	-20°C to -15°C
RNase free water	1mL x 1EA	-20°C to -15°C

4. PRECAUTIONS

- Immediately store the kit at -15°C to -20°C after receipt.
- Repeated freeze-thaw cycles can decrease the activity of this kit.
- The components of different batches may not be mixed and used
- Please store Primer & Probe Mixture (PM) should be protected from light. Especially, do not expose them to direct sun light.
- This kit can be used on various Real-Time PCR thermal cyclers that can detect the emitted fluorescence of the fluorescent dyes FAM, HEX and Texas Red.
- It is recommended to use powder-free gloves and change them frequently to avoid cross contamination.

5. EQUIPMENT REQUIRED BUT NOT PROVIDED

- RNA Extraction Kit (e.g. DIAKEY REALcheck Viral RNA/DNA Prep Kit)
- Table top micro centrifuge
- Vortex
- Micropipettes covering volumes of 1 uL to 1000 uL
- Centrifuge for PCR tubes or plates
- Real-Time PCR thermal cycler

6. ASSAY PROCEDURE

- Protocol of the kit consists of the following main workflow:

- Sample processing
- RNA preparation
- Reaction Set-up (qRT-PCR)
- Data Analysis – validity and qualitative result

- It is recommended to proceeding without interruption to avoid any degradation of isolated samples and reagents. If necessary, store isolated RNA at -70°C to -80°C before use and avoid repeated freezing and thawing.

7. PROTOCOL

(1) Sample processing

Sampling swabs are pooled in a sufficient volume of sterile buffer (e.g. normal saline or 0.1XTE) and soaked for an adequate period of time. Mix it thoroughly using vortex mixer and the supernatant is used for RNA isolation.

(2) RNA Preparation

Concentration of isolated RNA through DIAKEY REALcheck Viral RNA/DNA Prep kit or alternative RNA preparation kits should be between 1pg/uL and 1ug/uL. Ratio OD_{260/280} of isolated RNA should be 1.8 - 2.2 to ensure proper quality of the purified RNA.

(3) Reaction Set-up, Reverse Transcription and Amplification

- Perform vortexing for 1 - 2 sec. and spin down briefly before use of 2X OneStep qRT-PCR Master Mix (For probe), Primer & Probe Mixture (PM).
- Determine the total number of reactions through counting of the number of samples, NC(Negative Control) and PC(Positive Control).(e.g: total number of mixture for 20 rxns)

♦ Primer & Probe Mixture (PM): = Total 23 rxns
 $20 \text{ rxns}(\text{Number of samples}) + 1 \text{ rxn}(\text{Positive Control}) + 1 \text{ rxn}(\text{NC}) + 1 \text{ rxn}(\text{Pipetting error})$

- Prepare 23 Mixtures per each gene in consideration of the pipetting error and add it to each of the PCR Plate wells or PCR tubes. Then, add below templates each of them in the given order:

NC (D.W) → Sample RNA → PC (Positive Control)

♦ Primer & Probe Mixture (PM) Master Mix

Reagent	Volume (uL)	
	Per rxn	For 20rxns(total 23rxns)
2X onestep qRT-PCR Master Mix(for probe)	10.0	230.0
Primer & Probe Mixture(PM)	2.0	46.0
Total Master Mix	12.0	276, dispense 12uL per rxn
RNA (sample/NC/PC)		8.0
Total Reaction		20.0

► It is recommended to use ROX Reference Dye according to the characteristics of each Real-Time PCR thermal cycler

Add 8 uL of the NC and the sample RNA to the corresponding cavity and seal it individually, if possible. Then, 8 uL of PC are added to the corresponding cavity to minimize risk of potential cross-contaminations.

Place the cavities in the Real-Time PCR thermal cycler and run the test using the following parameters:

Step	Temperature	Duration
Reverse Transcription	50°C	30 mins
Activation of Polymerase	95°C	15 mins
Denaturation	95°C	10 sec
Annealing & Extension	62°C	40 sec
Fluorescence Detection	Channels FAM, HEX and Texas Red	45 Cycles

8. DATA ANALYSIS

Validity and Qualitative Result

The Real-Time RT-PCR test run is only valid if the FAM-curve & Texas Red-curve of NC corresponding to RdRp Gene and N Gene is negative (Ct>42) and the FAM-curve & Texas Red -curve of PC (positive Control, PC) corresponding to RdRp Gene and N is positive. FAM & Texas Red-Ct-Value of PC has to be under 35 for a valid test.

If the Ct value of HEX for the internal control, RNase P(RP) gene is 40 or less, it is determine that RNA is properly isolated from sample. However, it may not be detected depending on performance of commercialized viral RNA extraction kits used and please use the table presented below to verify the correct results.

* Real-Time PCR Test Analysis for 2019_nCoV (COVID-19)

Target Gene			NC		PC			Result Analysis
RdRp	N	RP	RdRp	N	RdRp	N	RP	
+	+	±	-	-	+	+	+	2019_nCoV(COVID-19) Positive
+	-	±	-	-	+	+	+	* Inconclusive Result
-	+	±	-	-	+	+	+	2019_nCoV(COVID-19) Negative
+	-	±	+	-	+	+	+	# Invalid Result
-	+	±	-	+	+	+	+	
+	+	±	+	+	+	+	+	
-	-	-	-	-	-	-	-	

PC should be positive and with CT value within 35 cycles. If PC are negative, the testing results for that plate are invalid. Repeat qRT-PCR test.

NC should be negative. If NCs are positive, the testing results for that plate are invalid.

9. USE PRECAUTION

- Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
- Do not smoke, eat or drink in areas where specimens or kit reagents are handled and do not pipette by mouth.
- Handle samples, reagents and laboratory equipment used for assay with extreme care, as they may potentially contain infectious agents.
- Avoid microbial contamination when the reagent vial be eventually opened or the contents be handled.
- Do not use reagents beyond the expiration date.
- Use only for In Vitro.

10. SYMBOL INFORMATION

Symbol	Used for
	Use-by date
	Serial number
	Catalog number
	Temperature limit
	Keep away from sunlight
	Contain sufficient for <n> tests
	Positive Control
	Caution
	Research use only

11. REFERENCES

- Emergency Use Authorization of In-Vitro Diagnostics for Infectious Disease, Division of Laboratory Diagnosis Management, CDC, Park Jae-Sun, Choi Young-sill, Yoo Cheon-Kwon
- Udiportal.mfds.go.kr/brd/view/P01_01?ntceSn=43
- Medical Device Act and Enforcement Decree of Medical Devices

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