WizDx™ COVID-19 Real-time PCR Kit

CE IVD
REF DX1200
Σ 100 Tests

INTENDED USE

WizDx™ COVID-19 Real-time PCR Kit is intended to be used to achieve qualitative detection of 2019-Novel Coronavirus (COVID-19) viral RNA extracted from nasopharyngeal swabs, oropharyngeal swabs, and sputum from patients.

PRINCIPLES OF THE TEST

WizDx™ COVID-19 Real-time PCR Kits provide components for one-step Real-time RT-PCR in a convenient format that is compatible with both rapid and standard qPCR cycling conditions.
WizDx™ COVID-19 Real-time PCR Kit uses dual-labeled hydrolysis probes that target two distinct regions in RdRP (RNA-dependent RNA polymerase) gene and E (Envelop) gene of SARS-CoV-2 genome. Detection probe for RdRP gene is labeled with FAM, the probe for E gene is labeled with HEX and the probe for Internal control is labeled with Cy5 reporter dye.

KIT STORAGE AND STABILITY

- This kit is stable at -20 ºC temperature.
- Wizbiosolutions does not recommend using the kit after the expiry date stated on the pack.
- Freeze/thawing should be avoided.

KIT CONTENTS

Component	Amount	Storage	Color
COVID-19 Detection Mix	1 vial	- 20ºC	
2X qRT-PCR Mix	1 vial	- 20ºC	
COVID-19 Positive Control	1 vial	- 20ºC	
RNase-Free Water	1 vial	- 20ºC	

REAGENT & EQUIPMENT TO BE SUPPLIED BY THE USER

- Real-Time PCR Instrument (CE validated device)
- Micropipette & sterile pipette tips
- Vortex mixer & microcentrifuge
- Protective ware and Disposable gloves

TEST SAMPLE

- Sputum, oropharyngeal swab, and nasopharyngeal swab samples

SAMPLE COLLECTION, STORAGE AND TRANSPORTATION

- Collect samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

QUALITY CONTROL

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizDx™ COVID-19 Real-time PCR PCR Kit is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTION

- Carefully read this instruction before starting the procedure.
- Only use by authorized person.
- Use only for in vitro diagnostic purpose.
- Do not use any reagent after the expiration date.
- Always wear personal protective equipment (gloves and a mask) when handling biohazardous agents in compliance with national regulations.
- Always use sterile, filtered pipette tips.
- Take care of the handling of the specimen to minimize the risk of infection.
- Dispose of waste in compliance with national or regional regulations after the test.

PROTOCOL

1. RNA Preparation

Different brand RNA extraction kits are available. For the RNA extraction, please follow the manufacturer's instructions. The recommended extraction kit is WizPrep™ Viral DNA/RNA Mini Kit (REF. W73050, manufactured by Wizbiosolutions Inc.)

2. Prepare the reaction mixture

Component	Sample	PTC	NTC
2X qRT-PCR Mix	10 ul	10 ul	10 ul
COVID-19 Detection Mix	5 ul	5 ul	5 ul
Sample (RNA)	5 ul		
COVID-19 Positive Control		5 ul	
RNase-Free Water			5 ul
Total	20 ul	20 ul	20 ul

* PTC : Positive template control, NTC : Non-template control

3. Real-time PCR program set-up

- Prepare appropriate qPCR tubes and labels. Additional qPCR tubes for positive control & negative control.

Step	Temp.	Time	Cycle
cDNA Synthesis	50 °C	15 min.	1
Initial Denaturation	95 °C	5 min.	1
Denaturation	95 °C	15 sec	40
Annealing (Probe detection)	60 °C	60 sec	

4. Fluorescence probe setting

Fluoresence	Target	Detection
FAM	RdRP gene	COVID-19 (SARS-CoV-2)
HEX	E gene	Beta coronavirus
Cy5	Internal Control	

5. Analysis of Results

For interpretation, please refer to the Interpretation table.

- Cut-off value of sample or positive control : ***Ct <37***

Note: Interpretation of sample results can be determined provided positive and negative control reactions are performed. The positive control must yield a signal in the FAM, HEX, and Cy5 channels. The Negative Control may show a Cy5 fluorescence signal but no FAM and HEX signal.

INTERPRETATION TABLE

Test	FAM (RdRP)	HEX (E)	Results
PTC	+	+	Valid
NTC	–	–	Valid
#1	+	+	The specimen is COVID-19 positive
#2	+	–	Retest; If still same result, COVID-19 positive
#3	–	+	Retest; If RdRP(-), E gene (+), the specimen might be other type of Beta coronavirus positive.
#4	–	–	Below the detection limit or negative

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PERFORMANCE EVALUATION

The results for the Coronavirus (COVID-19) CE IVD assay performance evaluation have been generated on the Bio-RAD CFX96 Touch Real-Time PCR Detection System with additional testing for analytical sensitivity (LoD).

1. Limit of Detection (LoD)

To analyze the "Limit of Detection" of WizDx™ COVID-19 Real-time PCR Kit, in vitro-transcribed RNA standard of E gene and RdRp gene of new coronavirus (2019-nCoV) were prepared. Prepared RNA standard were diluted to concentration as values in table below. Testing for each concentration was repeated 25 times.

RdRP gene

Conc. (Copy number)	10^5	10^4	10^3	10^2	$5x10^2$	$5x10^1$
Number of Testing	25	25	25	25	25	25
Number of positive	25	25	25	25	25	25
Detection rate (%)	100	100	100	100	100	100
Conc. (Copy number)	$1.25x10^2$	$6.25x10^1$	$3.13x10^1$	$1.56x10^1$	$7.81x10^0$	$3.91x10^0$
Number of Testing	25	25	25	25	25	25
Number of positive	25	25	25	25	8	2
Detection rate (%)	100	100	100	100	32	8
95% Probit analysis	$1.53x10^1$					

E gene

Conc. (Copy number)	10^5	10^4	10^3	10^2	$5x10^2$	$5x10^1$
Number of Testing	25	25	25	25	25	25
Number of positive	25	25	25	25	25	25
Detection rate (%)	100	100	100	100	100	100
Conc. (Copy number)	$1.25x10^2$	$6.25x10^1$	$3.13x10^1$	$1.56x10^1$	$7.81x10^0$	$3.91x10^0$
Number of Testing	25	25	25	25	25	25
Number of positive	25	25	25	25	3	0
Detection rate (%)	100	100	100	100	12	0
95% Probit analysis	$1.44x10^1$					

Results were statistically analyzed with probit analysis. LOD is determined as the lowest RNA concentration that can be detected more than 95%. LoDs of WizDx™ COVID-19 Real-time PCR Kit of RdRp gene and E gene were 15.3 copies/rxn and 14.4 copies/rxn respectively.

2. Interference

In order to evaluate the PCR reaction interference effect of substances and drug components contained in the specimen, three times repeated interference effect test was performed with three different concentrations (10^6, 10^4, 10^2) of in vitro-transcribed RNA standard of E gene and RdRp gene of new coronavirus (2019-nCoV) with or without interference substance. Results of interference effect test showed that WizDx™ COVID-19 Real-time PCR Kit was not affected by interference substance (Table below)

Interference Substances	Conc.	Category	Conc. (Copy number)					
			RdRP			E		
			10^6	10^4	10^2	10^6	10^4	10^2
Not included (D.W)	N/A	Aver.	21.4	28.5	34.9	22.0	28.8	35.1
		SD	0.12	0.05	0.02	0.03	0.09	0.09
Blood	5 %	Aver.	21.5	28.6	34.9	22.1	28.9	35.1
		SD	0.10	0.07	0.09	0.12	0.06	0.13
Mupirocin	7 ㎎/㎖	Aver.	21.4	28.5	34.9	22.0	28.9	35.1
		SD	0.04	0.10	0.09	0.04	0.06	0.03
Zanamivir	4 ㎎/㎖	Aver.	21.4	28.5	35.0	22.0	28.9	35.1
		SD	0.02	0.04	0.06	0.13	0.05	0.08
Nasal spray	10 %	Aver.	21.5	28.5	34.9	22.1	28.9	35.2
		SD	0.15	0.05	0.07	0.03	0.05	0.12

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3. Cross-reactivity

To prevent the cross-reaction of WizDx ™ COVID-19 Real-time PCR Kit, specific primers and probes to the new coronavirus (2019-nCoV) genes (E gene and RdRp gene) were designed and produced. And cross-reactivity was assessed using the sequence information of viruses associated with respiratory disease registered in GenBank (http://www.ncbi.nlm.nih.gov/). Using the WizDx ™ COVID-19 Real-time PCR Kit, each of the respiratory virus species, resident flora, and other strains was tested. The list of viruses and strains used for the cross-reaction is shown in the table below. The results from tests three times repeated show that there is no cross-reactivity.

No.	Target	Results	
		RdRP	E
1	Coronavirus RdRp	+	-
2	Coronavirus E	-	+
3	Influenza virus A	-	-
4	Influenza virus B	-	-
5	Adenovirus	-	-
6	Coronavirus NL-63	-	-
7	Enterovirus	-	-
8	Measles virus D9	-	-
9	Respiratory syncytial virus	-	-
10	Rhinovirus A01	-	-
11	Lactobacillus gasseri	-	-
12	Haemophilus parainfluenzae	-	-
13	Bordetella perusiss	-	-
14	Corynebacterium jeikeium	-	-
15	Legionella pneumophila	-	-
16	Neisseria meningitides	-	-
17	Pseudomonas aeruginosa	-	-
18	Staphylococcus epidermidis	-	-
19	Streptococcus pneumoniae	-	-
20	Streptococcus pyogenes	-	-
21	Streptococcus salivarius	-	-
22	Human	-	-

4. Clinical performance study

To evaluate the clinical performance of the of WizDx™ COVID-19 Real-time PCR Kit by comparing to PowerChek™ 2019-nCoV Real-time PCR kit (K company, Korea), the WizDx™ COVID-19 Real-time PCR Kit and the PowerChek™ 2019-nCoV Real-time PCR kit were tested and compared using RNAs extracted from nasopharyngeal swabs of COVID-19 patients (Korea University Guro Hospital, Korea). The result is in the table below.

		K company		Total
		Positive	Negative	
WizDx™ COVID-19 Real-time PCR Kit	Positive	2	0	2
	Negative	0	5	5
Total		2	5	7

Clinical Sensitivity: 100%(2/2), Clinical Specificity: 100% (5/5)

5. Precision (Repeatability, Reproducibility)

To analyze the repeatability and reproducibility WizDx™ COVID-19 Real-time PCR Kit, three repeated test was performed with three different concentrations (10^6, 10^4, 10^2) of in vitro-transcribed RNA standard of E gene and RdRp gene of new coronavirus (2019-nCoV) on the different conditions (three operators (Inter-Operator), (two instruments (Inter-Instrument), two sites (Inter-sites), three lots (Inter-lot)), two times per day for twenty-days. The statistical analysis of results is shown in the table below

Assay	Category	Conc. (Copy number)					
		RdRP			E		
		10^6	10^4	10^2	10^6	10^4	10^2
Inter-Operator	Aver.	21.3	28.5	34.9	22.1	28.8	35.1
	SD	0.08	0.11	0.09	0.08	0.11	0.07
Intra-Instrument	Aver.	21.3	28.4	34.9	22.1	28.9	35.1
	SD	0.07	0.11	0.10	0.08	0.10	0.10
Inter-site	Aver.	21.4	28.4	34.9	22.1	28.9	35.0
	SD	0.10	0.10	0.09	0.07	0.09	0.09
Inter-lot	Aver.	21.3	28.4	35.0	22.1	28.9	35.1
	SD	0.06	0.09	0.10	0.08	0.11	0.06

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TROUBLESHOOTING GUIDE

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls	• Incorrect detection channel has been chosen.	• Set Channel settings to FAM and Cy5
	• Pipetting errors	• Check for correct reaction setup. Repeat the PCR run.
	• No data acquisition programmed.	• Check the cycle programs
No signal increase in channel Cy5 is observed	• Inhibitory effects of the sample material (e.g., caused by insufficient purification). • Inappropriate storage of kit components.	• Use the recommended DNA preparation kit to purify template DNA. • Dilute samples or pipet a lower amount of sample DNA • Store the kit at -20 °C, protected from light and moisture
Fluorescence intensity is too low	• Low initial amount of target DNA.	• Increase the amount of sample DNA. • Exchange all critical solutions.
Negative control samples are positive.	• Carry-over contamination.	• Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. • Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies	• Insufficient centrifugation of the PCR strips. Resuspend PCR mix is still in the upper part of the vessel.	• Centrifuge PCR strips.
	• Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the vessels and seal

ORDERING INFORMATION

Product	Cat No.	Package
WizDx™ COVID-19 Real-time PCR Kit	DX1200	100 Test
WizPrep™ Viral DNA/RNA Mini Kit (V2)	W73052-100	100 Prep

SYMBOL GLOSSARY

Symbol	Meaning	Symbol	Meaning
REF	Catalogue Number		Manufacturer
LOT	Batch code	EC REP	Authorized representative in the European Community
	Temperature limit	IVD	In-Vitro Diagnostic Medical Device
	Use-by date	CE	CE Marking
Σ	Contents sufficient for <n> tests		Consult instructions for use
	Do not reuse		Keep away from sunlight

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