WizDx™ COVID-19 CrystalMix™ Multi PCR Kit

CE IVD
REF DX1203A
Σ 96 Tests

INTENDED USE
WizDx™ COVID-19 CrystalMix™ Multi PCR Kit is intended to be used to achieve qualitative detection of Severe acute respiratory syndrome coronavirus, (SARS-CoV-2) viral RNA extracted from nasopharyngeal swabs, oropharyngeal swabs, and sputum from patients.

PRINCIPLES OF THE TEST
WizDx™ COVID-19 CrystalMix™ Multi PCR Kit combines all the reagents necessary for successful one-step real-time RT-PCR in a convenient individually aliquot and lyophilized in 8-strip qPCR tube.
WizDx™ COVID-19 CrystalMix™ Multi 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. The detection probe for RdRP gene is labeled with FAM and the probe for E gene is labeled with HEX reporter dye.

KIT STORAGE AND STABILITY
- Store at 4 ~ 25°C, Do not freeze.
- Expires 12 months from date of manufacture.
- Do not use once the cone-shape mix shrinks as dot-form.

KIT CONTENTS

Component	Amount	Cap Color
COVID-19 CrystalMix	96 tubes	
COVID-19 Positive control *	1 vial	
RNase-Free water *	1 vial	

* Before using the positive control, add 200µl of RNase-Free water and dissolve sufficiently before use.
* Positive control & RNase-Free water are shipped at room temperature. After receipt, please store in refrigerated or frozen.

REAGENT AND EQUIPMENT TO BE SUPPLIED BY THE USER
- CFX-96 Real-Time PCR System (Bio-Rad Laboratories, Inc.)
- 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
- Specimen collection assays are not provided with the assay. Refer to CDC guidelines for specimen collection and storage at: https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

QUALITY CONTROL
In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizDx™ COVID-19 CrystalMix™ Multi 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 healthcare professionals.
- 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

[Step 1] RNA Preparation
The WizDx™ COVID-19 CrystalMix™ Multi PCR Kit has been validated with the Thermo Fisher RNA extraction kit (Catalog #: A42359). For the RNA extraction, please follow the manufacturer's instructions.

[Step 2] Prepare PCR Reaction
1) Prepare the PCR reaction as the following table.

Component	Sample	PTC	NTC
COVID-19 CrystalMix tube	1 tube	1 tube	1 tube
Sample (RNA)	20 ul		
COVID-19 Positive Control		20 ul	
RNase-Free Water			20 ul
Total	20 ul	20 ul	20 ul

* PTC : Positive template control, NTC : Non-template control
☞ To avoid contamination, close the cap immediately after placing the sample in the tube.
2) Vortex for 10 ~ 30sec. and briefly spin down for 5sec.
3) Insert the CrystalMix tube to the Real-Time PCR System.

[Step 3] Run Real-time PCR
1) Prepare the Real-time PCR program set-up as the following table.

Step	Temp.	Time	Cycle
cDNA Synthesis	50 °C	15 min.	1
Initial Denaturation	95 °C	5 min.	1
Denaturation	95 °C	15 sec	45
Annealing (Probe detection)	60 °C	40 sec	

2) Fluorescence probe and threshold setting as the following table.

Fluoresence	Target	Threshold
FAM	RdRP	100
HEX	E	100
Cy5	Internal Control	100

[Step 4] Analysis of Results
For interpretation, please refer to the Interpretation table.
- Cut-off value of sample or positive control : ***Ct <40***

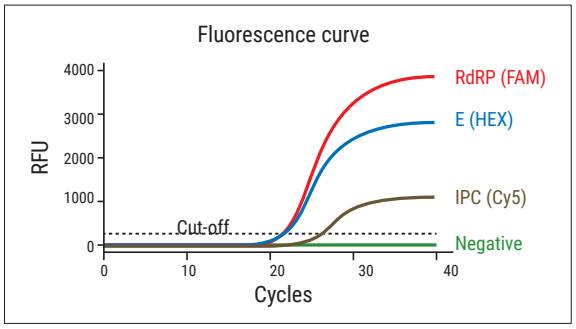
Test	FAM	HEX	Cy5	Results
#1	+	+	+/–*	COVID-19 detected
#2	+	–	+/–	COVID-19 detected
#3	–	+	+/–	Inconclusive COVID-19**
#4	–	–	+	No detected
#5	–	–	–	Invalid / Retest

Note:
* In case the target gene signal is strong, Cy5 (IPC) could be negative, and the result can be interpreted without IPC result.
** Depending on the sample condition, the sample has a possibility of COVID-19 positivity, recommend re-test.

WizDx™ COVID-19 CrystalMix™ Multi PCR Kit

CE IVD
REF DX1203A
Σ 96 Tests

EXAMPLE OF TEST RESULTS



PERFORMANCE EVALUATION

The results for the COVID-19 assay performance evaluation have been generated on the CFX96™ Real-Time PCR detection system (Bio-Rad Laboratories, Inc.) with additional testing for analytical sensitivity.

1. Limit of Detection (LoD)

To analyze the "Limit of Detection (LoD)" of WizDx™ COVID 19 CrystalMix™ Multi PCR Kit, a study was performed using SARS-CoV-2 RNA reference material, provided by Korea Research Institute of Standards and Science (KRISS) with serial dilution. Prepared RNA were spiked into clinical negative nasopharyngeal swab and sputum matrix. The viral RNA was extracted using WizPure™ Viral DNA/RNA Mini Kit (V2) (Wizbiosolutions Inc.) and amplified on CFX96 Real-Time PCR detection system. Testing of 6 different concentrations were repeated 25 times. Results were statistically analyzed with probit analysis. LOD is determined as the lowest RNA concentration that can be detected more than 95%. LoDs Limit of detection was estimated where 100% detection rate was confirmed on RdRP gene at 0.72 copies/ul, and E gene at 0.77 copies/ul.

RdRP gene	Nasopharyngeal swab			Sputum		
Conc. (copies/㎕)	Mean Ct	SD	Detection rate	Mean Ct	SD	Detection rate
50	30.30	0.31	25/25	30.29	0.38	25/25
5	33.63	0.54	25/25	33.69	0.47	25/25
1.44	35.67	0.33	25/25	35.69	0.44	25/25
1.08	35.95	0.57	25/25	36.04	0.32	25/25
0.72	36.48	0.38	25/25	36.90	0.43	25/25
0.36	37.40	1.01	20/25	37.98	0.85	18/25

E gene	Nasopharyngeal swab			Sputum		
Conc. (copies/㎕)	Mean Ct	SD	Detection rate	Mean Ct	SD	Detection rate
50	31.23	0.52	25/25	31.25	0.39	25/25
5	34.13	0.77	25/25	34.59	0.46	25/25
1.54	36.74	0.50	25/25	36.77	0.39	25/25
1.16	36.90	0.43	25/25	37.31	0.35	25/25
0.77	37.59	0.37	25/25	37.99	0.34	25/25
0.39	38.82	1.08	19/25	39.26	0.43	16/25

Results were statistically analyzed with probit analysis. LOD is determined as the lowest RNA concentration that can be detected by more than 95%.
The detection rate of WizDx™ COVID-19 CrystalMix™ Multi PCR Kit of RdRp and E were 0.72 copies/㎕ and 0.77 copies/㎕ 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 (500, 50, 5 copies/㎕) of reference materials of RdRp and E with or without interference substance. Results of the interference effect test showed that WizDx™ COVID-19 CrystalMix™ Multi PCR Kit was not affected by interference substance (Table below).

Interference Substances	Conc.	Category	Conc. (Copy number/㎕)					
			RdRP			E		
			500	50	5	500	50	5
Not included (D.W)	N/A	Aver.	25.92	29.25	32.87	27.15	30.42	33.92
		SD	0.13	0.12	0.23	0.36	0.24	0.26
		CV (%)	0.50	0.39	0.71	1.32	0.80	0.76
Nasal spray	0.1 %	Aver.	26.08	29.37	32.56	27.13	30.11	33.90
		SD	0.34	0.27	0.39	0.04	0.31	0.32
		CV (%)	1.31	0.93	1.21	0.15	1.05	0.93
Mucin	60 ㎎/㎖	Aver.	25.92	28.74	32.52	26.89	30.57	33.79
		SD	0.66	0.20	0.30	0.17	0.22	0.53
		CV (%)	2.55	0.69	0.92	0.65	0.72	1.58
Human gDNA	1 ng/ul	Aver.	26.20	30.01	32.65	26.20	30.01	32.65
		SD	0.29	0.49	0.44	0.29	0.49	0.44
		CV (%)	1.11	1.64	1.34	1.11	1.64	1.34
EDTA	0.02%	Aver.	26.12	29.42	33.40	27.41	30.49	33.71
		SD	0.33	0.51	0.43	0.37	0.25	0.42
		CV (%)	1.26	1.74	1.28	1.33	0.83	1.25
Human blood	2%	Aver.	25.94	29.64	33.80	26.87	30.05	33.83
		SD	0.12	0.33	0.56	0.35	0.14	0.56
		CV (%)	0.45	1.12	1.65	1.29	0.46	1.66

3. Cross-reactivity

To prevent the cross-reaction of the WizDx™ COVID-19 CrystalMix™ Multi PCR Kit, specific primers and probes to the RdRp and E 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 CrystalMix™ Multi PCR Kit, each of the respiratory virus species, resident flora, and other strains were 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 to show that there is no cross-reactivity.

No.	Target	Results	
		RdRP	E
1	SARS-CoV-2	+	+
2	SARS-coronavirus	-	+
3	MERS-coronavirus	-	-
4	Influenza virus B (Yamagata)	-	-
5	Influenza virus B (jangsu/10/2003)	-	-
6	Influenza A (pdm09/England/195/2009,H1N1)	-	-
7	Human Respiratory syncytial virus A2	-	-
8	Human coronavirus 229E	-	-
9	Human Norovirus GI	-	-
10	Norovirus GII	-	-
11	Adenovirus serotype 2	-	-
12	Human Cytomegalo virus	-	-
13	Epstein Barr virus	-	-
14	Human Herpes simplex virus type1	-	-
15	Human Herpes simplex virus type2	-	-
16	Parvo B19 virus	-	-
17	Varicella zoster virus (type B)	-	-
18	Adeno virus (Type 1)	-	-
19	Entero virus	-	-
20	Measles virus	-	-
21	Human Respiratory syncytial virus B	-	-
22	Rhinovirus	-	-
23	Human coronavirus NL-63 (HCoV-NL63/CN0601/14)	-	-
24	Lactobacillus gasseri	-	-
25	Haemophilus parainfluenzae	-	-
26	Bordetella perusiss	-	-
27	Corynebacterium jeikeium	-	-
28	Legionella pneumophila	-	-
29	Neisseria meningitides	-	-
30	Pseudomonas aeruginosa	-	-
31	Staphylococcus epidermidis	-	-
32	Streptococcus pneumoniae	-	-
33	Streptococcus pyogenes	-	-
34	Streptococcus salivarius	-	-
35	Human RNA	-	-

4. Clinical performance study

To evaluate the clinical performance of the WizDx™ COVID-19 CrystalMix™ Multi PCR Kit by comparing to S company detection kit, the WizDx™ COVID-19 CrystalMix™ Multi PCR Kit and the S company detection kit were tested and compared using RNAs extracted from oropharyngeal, nasopharyngeal swabs, and sputum of SARS-CoV-2 patients' left-over archived samples. The result is in the table below.

Oropharyngeal and nasopharyngeal swab		S company		Total
		Positive	Negative	
WizDX™ COVID-19 CrystalMix™ Multi PCR Kit	Positive	80	1	81
	Negative	0	129	129
Total		80	130	210

Diagnostic Sensitivity: 100% (95% CI 95.42% ~ 100%)
Diagnostic Specificity: 99.23% (95% CI 95.77% ~ 99.86%)
Positive percent agreement: 100% (95% CI 95.42% ~ 100%)
Negative percent agreement: 99.23% (95% CI 95.77% ~ 100%)

Sputum		S company		Total
		Positive	Negative	
WizDX™ COVID-19 CrystalMix™ Multi PCR Kit	Positive	79	1	80
	Negative	1	129	130
Total		80	130	210

Diagnostic Sensitivity: 98.75% (95% CI 93.25% ~ 99.78%)
Diagnostic Specificity: 99.23% (95% CI 95.77% ~ 99.86%)
Positive percent agreement: 98.75% (95% CI 93.25% ~ 99.78%)
Negative percent agreement: 99.23% (95% CI 95.77% ~ 99.86%)

Total		S company		Total
		Positive	Negative	
WizDX™ COVID-19 CrystalMix™ Multi PCR Kit	Positive	159	2	161
	Negative	1	258	259
Total		160	360	420

Diagnostic Sensitivity: 99.38% (95% CI 96.55% ~ 99.89%)
Diagnostic Specificity: 99.23% (95% CI 97.24% ~ 99.79%)
Positive percent agreement: 99.38% (95% CI 96.55% ~ 99.89%)
Negative percent agreement: 99.23% (95% CI 97.24% ~ 99.79%)
Total percent agreement: 99.29% (95% CI 97.92% ~ 99.76%)

5. Precision (Repeatability, Reproducibility)

To analyze the repeatability and reproducibility WizDx™ COVID-19 CrystalMix™ Multi PCR Kit, three repeated tests were performed with three different concentrations of reference materials of RdRp and E on the different conditions, three operators (Inter-Operator), 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		
		1.44	1.08	0.72	1.54	1.16	0.77
Inter-Operator	Aver.	35.67	35.92	36.54	36.74	36.82	37.64
	SD	0.32	0.45	0.53	0.46	0.42	0.61
	CV (%)	0.91	1.26	1.45	1.25	1.15	1.61
Inter-site	Aver.	35.80	35.86	36.51	36.78	36.81	37.66
	SD	0.36	0.46	0.61	0.46	0.40	0.58
	CV(%)	1.02	1.29	1.68	1.26	1.10	1.53
Inter-lot	Aver.	35.61	35.89	36.59	36.78	36.82	37.72
	SD	0.36	0.51	0.40	0.50	0.45	0.47
	CV(%)	1.00	1.43	1.10	1.36	1.22	1.25

WizDx™ COVID-19 CrystalMix™ Multi PCR Kit

CE IVD
REF DX1203A
Σ 96 Tests

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 HEX
	• Pipetting errors	• Check for correct reaction setup. Repeat the PCR run.
	• No data acquisition programmed.	• Check the cycle programs
Fluorescence intensity is too low	• Low initial amount of target nucleic acid	• Increase the amount of sample nucleic acid. • 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 CrystalMix™ Multi PCR Kit	DX1203A	96 Tests
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

MFDS Registered No. : 6289
Product License No. : IVD-21-369

Wizbiosolutions Inc.
A-802, Woolim Lions Valley 2, Sagimakgol-ro 45beon-gil 14
Jungwon-gu, Seongnam, 13209 South Korea
TEL : +(82) 70-7605-5066 FAX : +(82) 31-624-3066
sales@wizbiosolution.com www.wizbiosolution.com

EC REP **MT Promedt Consulting GmbH**
Altenhofstrasse 80
66386 St. Ingbert, Germany

FQP-806-01-92
(REV. 1.0.1, 29.09.21)