RADI COVID-19 Detection Kit

REF RV008

1. Intended use

The RADI COVID-19 Detection Kit is an in vitro diagnostic medical device, based on realtime RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA).
It is intended for the presumptive qualitative detection of nucleic acid from the COVID-19 in upper and lower respiratory specimens.
The assay is for use by a laboratory professional trained to use real-time PCR in a laboratory.

2. Summary and Explanation

The SARS-CoV-2 which is the causative agent for an outbreak (COVID-19) first reported on 31 Dec 2019 in China, is a new beta coronavirus. Infection with COVID-19 causes respiratory symptoms, fever, fatigue and in severe cases, pneumonia, severe acute respiratory syndrome, organ failure and even death.

The RADI COVID-19 Detection Kit is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis COVID-19 and in based on widely used nucleic acid amplification technology.

3. Kit Components

Materials Provided (100 tests/kit)

Lid Color of Tube	Component	Volume (㎕)
Brown	Primer & Probe mixture	500
Yellow	3X RT MasterMix	1000
Red	Positive control	300
Blue	RNase free water	1000

Materials Required BUT NOT PROVIDED

- Micropipette
- Sterilized pipette tips with filter barriers
- Centrifuge, Vortex mixer
- Disposable powder-free gloves
- Real Time PCR machine
  - RADI Cycler - MyGo Mini
  - CFX96 Real-Time PCR Detection System
  - Applied Biosystems 7500 Real-Time PCR System
- Consumables relating the RT-PCR

4. Storage

- All components should be stored between -25°C and -15°C upon arrival.
- Storage between +2°C and +8°C should not exceed a period of two hours.

5. Specimen

- Upper and lower respiratory specimens such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate.
- Follow specimen collection devices manufacturer instructions for proper collection method.

6. Extraction

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kit are recommended in combination with the RADI COVID-19 Detection Kit for nucleic acid extraction:

- RADI Prep plus, KH Medical, Republic of Korea
- QIAmp Viral RNA Mini Kit, Qiagen

7. PCR Mixture Protocol

Component		Volume (㎕)
PCR Mixture	3X RT MasterMix	10
	Primer & Probe Mixture	5
Extracted RNA, PC, NTC		15
Total Volume		30

① Pipette 15 ㎕ of above mixes(PCR Mixture) into each well, according to your qPCR experimental tube set up.
② Pipette 15 ㎕ of extracted RNA, into each well, according to your experimental tube set up.
③ For positive well, add 15 ㎕ of Positive Control.
④ For negative well, add 15 ㎕ of RNase free water.

8. PCR amplification Protocol

Temperature	Time	Cycle
50 ℃	20 min	1
95 ℃	5 min	1
95 ℃	10 sec	45
55* ℃	30 sec	

* Fluorogenic data should be collected during this step through the FAM and VIC channels.

9. Fluorescence Detector

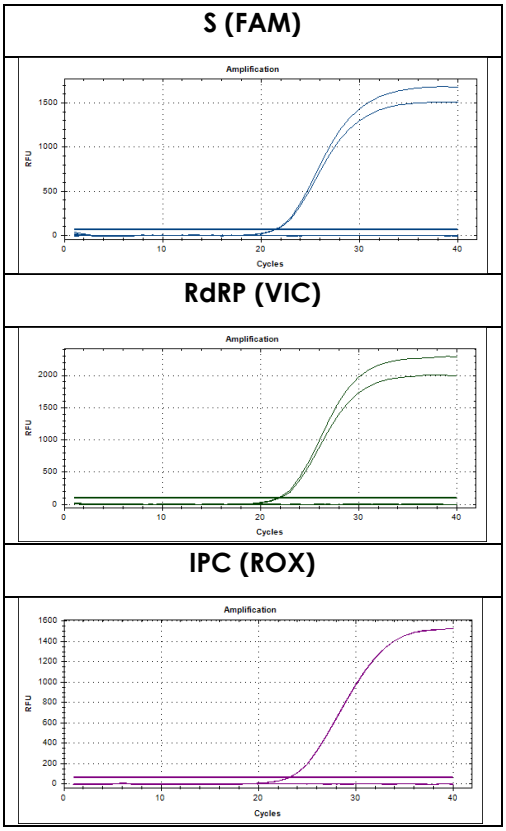
Target	Reporter
S gene	FAM
RdRP gene	VIC
IPC(Internal Control)	ROX

10. Precautions

1) Do not use the kit after its expiration date.
2) This assay needs to be carried out by skilled personnel.
3) Prepare quickly the Reaction mix above ice or in the cooling block.
4) Before using, please check the integrity of the reagent tubes, and remember to mount the spin tips into the appropriate position.
5) Please wear a mask and disposable gloves when handling.
6) Use always sterile pipette tips with filters.
7) Avoid eyes, skin and clothing contact with reagents. In case of any contact, flush with flowing water.

11. Result

<Data from CFX96>



The threshold is 1/20 of maximum delta Rn.

※ Threshold can be set up differently depending on the machine to use.

12. Data analysis

Example	Target			Result
	S (FAM)	RdRP (VIC)	IPC (ROX)	
1	≤40	≤40	≤40	Positive
2	≤40	≤40	—	Positive
3	≤40	—	≤40	Positive
4	≤40	—	—	Positive
5	—	≤40	≤40	Positive
6	—	≤40	—	Positive
7	—	—	≤40	Negative
8	—	—	—	Invalid
9	40~45	40~45	≤40	Invalid
10	40~45	—	≤40	Invalid
11	—	40~45	≤40	Invalid

13. Performance characteristics

Analytical sensitivity (Limit of Detection)

To test the sensitivity and limit of detection(LOD) of RADI COVID-19 Detection kit, synthesized RNA by *in vitro* transcription was diluted to 4 concentrations and ran 24 times for each.

LOD is 0.66 copies/㎕.

Target	Copies/㎕	Mean Ct	Result in agreement	Percent agreement
S gene	1.66	37.62	24/24	100 %
	0.66	39.88	23/24	95.83 %
	0.5	40.18	24/24	100 %
	0.33	39.57	20/24	83.32 %
RdRP gene	1.66	36.55	24/24	100 %
	0.66	38.06	23/24	95.83 %
	0.5	39.08	22/24	91.66 %
	0.33	38.47	21/24	87.49 %

*IPC mean Ct : 22.93

Cut off Value

Limit of detection was decided as the cutoff value. A Ct value of 40 was set as the cut-off of RADI COVID-19 Detection KIT.

Cross Reactivity

RADI COVID-19 Detection KIT did not cross-react with any of following pathogens.

	No.	Cross Reactivity pathogens	Conc.
NCCP	14547	Salmonella Enteritidis	75ng
NCCP	16207	Salmonella Typhimurium	75ng
NCCP	15758	Salmonella enterica	75ng
NCCP	14641	Salmonella Typhi	75ng
NCCP	13713	Vibrio parahaemolyticus	75ng
NCCP	12843	Vibrio cholera	75ng
NCCP	16296	Bacillus cereus	75ng
NCCP	16297	Bacillus subtilis	75ng
NCCP	15938	Bacillus infantis	75ng
NCCP	15871	Staphylococcus aureus	75ng
NCCP	14669	Staphylococcus hominis	75ng
NCCP	11192	Campylobacter jejuni	75ng
NCCP	14714	Listeria monocytogenes	75ng
NCCP	15661	Escherichia coli	75ng
NCCP	15911	Clostridium perfringens	75ng
NCCP	12713	Yersinia enterocolitica	75ng
NCCP	16366	Clostridium tertium	75ng
NCCP	11820	Clostridium difficile	75ng
NCCP	16330	Salmonella Stanley	75ng
NCCP	14708	Haemophilus parainfluenzae	75ng
NCCP	14675	Haemophilus haemolyticus	75ng
NCCP	14785	Haemophilus influenzae	75ng
NCCP	13753	Neisseria meningitidis	75ng
NCCP	15882	Streptococcus pneumoniae	75ng
NCCP	43193	Adenovirus	52.5ng
NCCP	43248	Dengue virus	52.5ng
NCCP	43280	Zika virus	52.5ng
NCCP	43132	Chikungunya virus	52.5ng
NCCP	41308	Japanese Encephalitis virus	52.5ng
NCCP	43165	Enterovirus	52.5ng
NIBSC	15/222	EBOV RNA NP-VP35-GP	52.5ng
NIBSC	06/202	HPV 16 DNA	52.5ng
NIBSC	06/206	HPV 18 DNA	52.5ng
NCCP	40204	Measles virus	75ng
NCCP	41205	Coxsackievirus	75ng
NCCP	43221	Coxsackievirus	75ng
NCCP	43225	Rhinovirus	75ng
NCCP	43230	Influenza A virus (H3N2)	75ng
NCCP	43231	Influenza A virus(H1N1)	75ng
NCCP	43232	Influenza B virus	75ng
NCCP	43238	Respiratory Syncytial virus A	75ng
NCCP	43239	Respiratory Syncytial virus B	75ng

NCCP	43281	Vaccinia virus	75ng
NCCP	43214	human Coronavirus NL63	75ng
NCCP	72002	Legionella pneumophila	52.5ng
NCCP	72026	Bordetella pertussis	30ng
NCCP	72006	Pseudomonas aeruginosa	75ng
NCCP	72077	Mycobacterium tuberculosis	22.5ng
NCCP	72059	Staphylococcus epidermidis	30ng
NCCP	43261	SFTS virus	52.5ng
NCCP	43108	Herpes Simplex virus 2	52.5ng
NCCP	43110	Herpes Simplex virus 1	52.5ng
NCCP	41003	Echovirus	52.5ng

Reproducibility

Four replicates of three concentrations test were made by two different experimenters.
Each test was repeated twice with the RADI COVID-19 Detection kit.
Mean Ct value and CV is calculated and All CV values are below 2%.

Target	Concentrations	Mean Ct	SD	CV
S gene	LOD	38.32	0.71	1.84
	3x LOD	36.54	0.55	1.49
	5x LOD	35.75	0.46	1.28
RdRP gene	LOD	37.61	0.66	1.74
	3x LOD	36.08	0.44	1.23
	5x LOD	35.10	0.24	0.69

*IPC mean Ct : 22.88

Repeatability

To test the repeatability of RADI COVID-19 Detection kit, synthesized RNA by *in vitro* transcription was diluted to three concentrations.
Test runs of three concentrations were made in four replicates for 15 days using 3 different lots of the RADI COVID-19 Detection Kits.
Mean Ct value and CV of Between Lot, Between Day and Between Run is calculated.
All CV values are below 3%.

	Target	Concentration	Mean Ct	SD	CV
Between Lot	S gene	LOD	38.18	0.80	2.09
		3x LOD	36.25	0.44	1.22
		5x LOD	36.08	0.60	1.67
	RdRP gene	LOD	37.81	0.84	2.21
		3x LOD	35.78	0.30	0.84
		5x LOD	35.52	0.47	1.32

*IPC mean Ct : 22.84

	Target	Concentration	Mean Ct	SD	CV
Between Day	S gene	LOD	38.39	1.04	2.72
		3x LOD	36.39	0.55	1.52
		5x LOD	35.65	0.47	1.30
	RdRP gene	LOD	37.61	0.83	2.21
		3x LOD	35.66	0.46	1.30
		5x LOD	34.92	0.48	1.37

*IPC mean Ct : 22.77

	Target	Concentration	Mean Ct	SD	CV
Between Run	S gene	LOD	38.60	0.69	1.79
		3x LOD	36.50	0.51	1.40
		5x LOD	35.42	0.44	1.23
	RdRP gene	LOD	37.97	1.12	2.95
		3x LOD	35.50	0.37	1.04
		5x LOD	34.66	0.37	1.08

*IPC mean Ct : 22.67

Description of Symbol Used

Symbol	Description	Symbol	Description
REF	Catalogue number		Consult instruction for use
LOT	Lot number	-25℃ -15℃	Storage at -25℃ to -15℃
	Use by date		Manufacturer
Σ	Contains sufficient for tests	CE	CE mark
IVD	In vitro diagnostic Medical Device	EC REP	Authorized representative in the European community

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