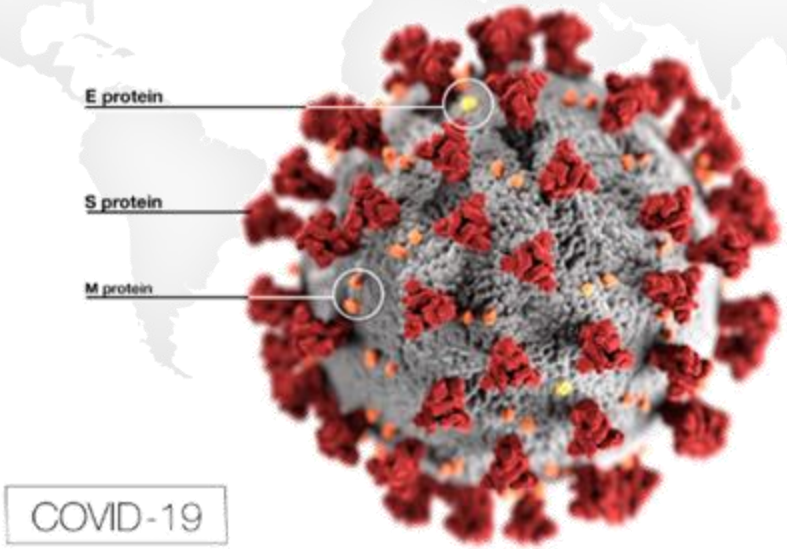
RADI COVID-19 Detection Kit



Agenda

About KH Medical Co. ltd

About RADI COVID-19 Detection KIT

1. Introduction
2. Procedure of Diagnostics
3. Diagnostic Kit Components
4. Prepare qPCR Mixture
5. Result Analysis
6. Product Performance_FIND

About RADI PREP Swab and Stool DNA/RNA KIT

1. Introduction
2. Reagent Components
3. Performance
4. Training and maintenance support

SARS-COV-2

- RNA(single-stranded RNA)
- Sequence length : 29,990 base pairs
- Genes : orf1ab, S, orf3a, E, M, orf6, orf7a, orf8, N, orf10

RADI COVID-19 Detection KIT

- Detection of **S gene** and **RdRP gene(=Orf1 gene)**
- Product Design : Compared SARS-COV-2 and other Corona viruses for homology.
- Under 75% of homology gene were selected

RADI COVID-19 Detection KIT_Test Procedure

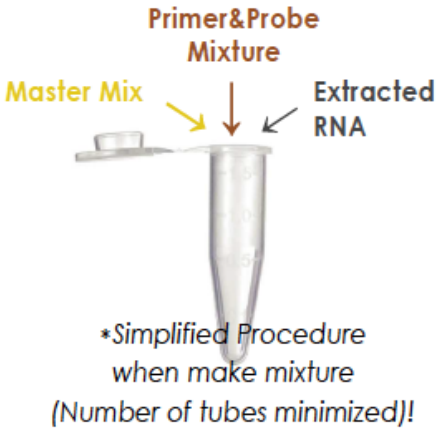
(KH Medical provide Total Solution for COVID-19 Detection: From Extraction to PCR)

Test Procedure

RADI PREP Swab and Stool DNA/RNA Kit (KH Medical)

QIAamp DSP Viral RNA Mini Kit (KH Medical)

QIAamp Viral RNA Mini Kit (KH Medical)



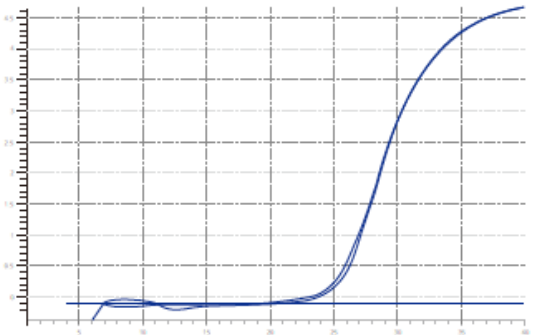
RADI mini cycler,16, or 96

CFX96(BioRad)

ABI7500(ThermoFisher)

Rotor-Gene Q (Qiagen)

LightCycler 480(Roche)



Nucleic acid extraction → *Make Mixture → Real-time PCR Run → Analysis

RADI PREP-PLUS

RADI CYCLER

our TAT of 80 minutes and test up to 576 samples if using the RADI 96 in an 8hr shift

RADI COVID-19 Detection KIT

RADI COVID-19 Detection Kit	
Intended use	Qualitative detection of COVID-19
Specimen type	RNA extracted in nasal swab or Sputum
Target	RdRP, S
Product contents	• 3X RT MasterMix (Yellow) • COVID-19 Primer & Probe Mixture (Brown) • COVID-19 positive control (Red) • RNase free water (Blue)
LoD	0.66 copies/μℓ
Storage condition	-25°C ~ -15°C

Diagnostic KIT Components

Kit Contents
1Kit = 100 Tests

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

➢ Compatible Real Time PCR Machines

- **RADI Cyclers (mini, 16 and 96)**
- **CFX96 Real-Time PCR Detection System, Bio-rad**
- **Applied Biosystems 7500 Real-Time PCR System, ThermoFisher**
- **Rotor-Gene Q, QIAGEN**
- **LightCycler 480 System, Roche**

CFX96

ABI 7500

Rotor-Gene Q

LightCycler 480

Prepare qPCR Mixture

PCR Mixture protocol

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

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.

Fluorescence Detector

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

Prepare qPCR Mixture

1) Prepare the mixture for Real-time PCR reaction in the order below.

No	Material	Volume/Reaction
1	3X Master Mix	10ul
2	Primer & Probe Mixture	5ul
3	Template (Sample, PC or NC)	15ul
	Total	30ul

PCR Mixture

**** Calculate the PCR mixture amount like below:***

Volume/Reaction * N(Samples+PC+NC+1)

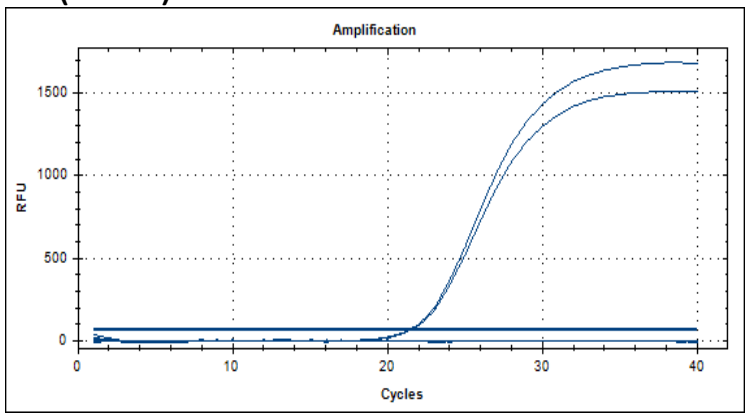
2) Aliquot 15ul of reaction mixture in each tube.

3) Add 15ul each of 1 negative control, 1 Positive control and number of prepared samples(Extracted RNA) into each tube.

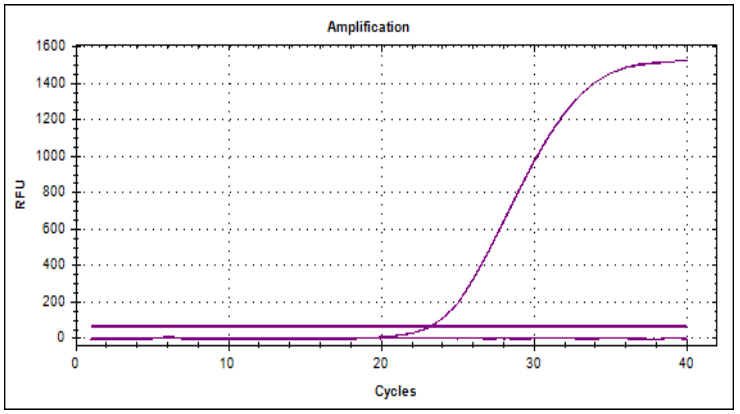
4) Run On PCR Machine.

Result Analysis

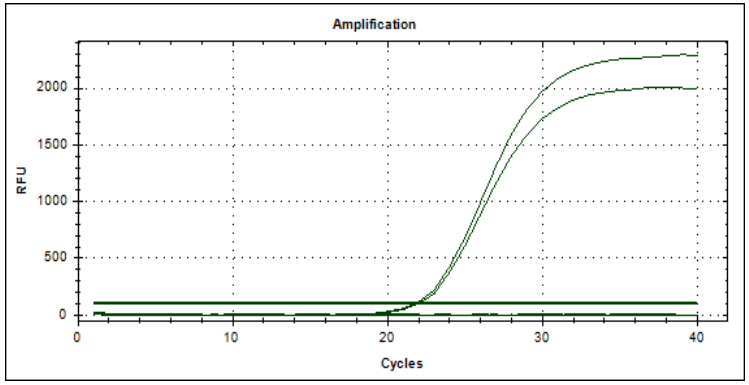
S (FAM)



IPC (ROX)



RdRP (VIC)



The threshold is 1/20 of maximum delta Rn.

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

Result 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

* No reaction of IPC :
Due to high concentration of RNA, dNTP is consumed for reaction.

* No sample(RNA) is added

* Reaction after 40Ct : Rerun

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%

Target	Copies/㎕	Mean Ct	Result in agreement	Percent agreement
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

Cross Reactivity

RADI COVID-19 Detection KIT did not cross-react with any of 53 pathogens.

Comparative Evaluation by using WHO published Primer & Probe

RADI COVID-19 Detection Kit's Primer & Probe design could detect up to 10 copies/rxn compared to WHO Primer & Probe which detect up to 10^2 copies/rxn for E gene and 10^3 copies/rxn for RdRP gene.

	RADI COVID-19 Detection KIT				WHO primer & probe			
Copies/rxn	S gene		RdRP gene		E gene		RdRP gene	
10^6	21.06	21.2	21.46	21.63	23.24	22.72	24.75	24.78
10^5	25.05	24.97	25.01	24.65	26.48	26.34	28.17	28.12
10^4	28.99	29.22	28.22	28.29	29.90	29.88	31.44	31.31
10^3	32.47	32.62	31.96	31.81	32.48	32.26	34.61	33.72
10^2	36.51	36.41	35.4	35.18	35.69	36.76	N/A	N/A
10	39.95	39.66	39.18	38.05	N/A	N/A	N/A	N/A

Product Evaluation

https://www.finddx.org/covid-19/sarscov2-eval-molecular/

FIND
Because diagnosis matters

KHMedical

Home > COVID-19 diagnostics > FIND evaluation update: SARS-CoV-2 molecular diagnostics

On 19 February 2020, FIND launched an expression of interest (EOI) for test developers of *in vitro* diagnostics (IVDs) that detect SARS-CoV-2 nucleic acid (molecular tests). The EOI closed on 9 March 2020. Over 200 submissions were received.

Applications were reviewed according to the following scoring criteria. The tests selected for the first round of independent evaluation are listed below. Additional tests will be included in subsequent rounds, including automated tests for use on closed/proprietary systems. Results of the evaluations will be posted in the coming weeks.

Manual (open) PCR tests included in the round 1 evaluation:

Company	Assay	Country of manufacturer	Target	Regulatory status
altona Diagnostics	RealStar® SARS-CoV-2 RT-PCR Kit 1.0	Germany	E gene and S gene	CE-IVD
Atila Biosystems Inc	Atila iAMP® COVID Detection Kit	USA	ORF1ab and N region	RUO
KH Medical Co. Ltd.	RADI COVID-19 Detection Kit (RV008)	Rep. of Korea	ORF1ab gene and S gene	CE-IVD
	PCR kit for detecting 2019-nCoV			
bioMérieux SA	SARS-COV-2 R-GENE®	France	N gene and RdRp gene	RUO

Product Evaluation - Results

FIND EVALUATION UPDATE: SARS-COV-2 MOLECULAR DIAGNOSTICS

LOD analysis performed using cultured viral stocks from a clinical isolate from Switzerland

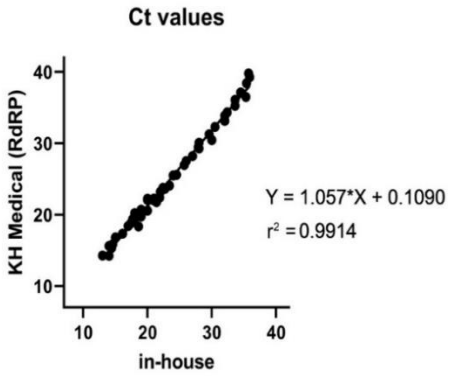
Clinical Performance conducted on extracted samples from patients suspected to have COVID-19 who were tested using a validated in-house PCR method from 26 Feb 2020 to 26 March 2020

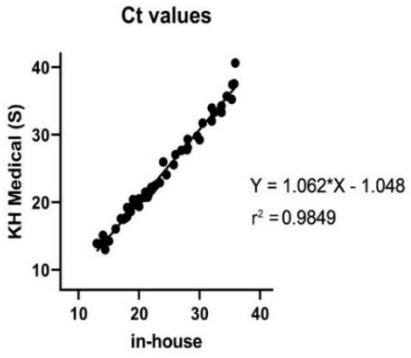
Testing done at HUG; Comparator Assay = In-house assay optimized from TIB MOLBIOL assay

Company	Gene Target	Verified LOD (copies/reaction)	Avg Ct (lowest dilution 10/10)	Clinical Sensitvity (50 positives)	Clinical Specificity ** (100 negatives)	Product No.	Product Name	Lot No.	PCR Platform	Supplier recommended Ct Cutoff
KH Medical Co. Ltd.	S	1-10	37.94	100%	100%	RV008	RADI COVID-19 Detection Kit	V008.200202	BioRad CFX96 deep well	≤ 40
	RdRP	10-50	36.74	100%	100%					

*** note: further investigation can be considered to determine if true false positives or whether originally a false negative*

RV008 RADI COVID-19 Detection Kit; LOT V008.200202





Certification

- CE IVD
- ISO 13485:2016
- ANVISA
- Kenya PPB
- FDA EUA pending
- WHO EUAL Pending
- Tanzania TMDA pending

About RADI PREP Swab and stool DNA/RNA KIT
(Manual KIT)

What is a RADI PREP Swab and stool DNA/RNA KIT

The RADI PREP Swab and Stool DNA / RNA KIT is a reagent used to extract nucleic acids from bacteria and viruses present in samples collected from, human feces, nasopharyngeal swab, Oropharyngeal swab, sputum. The method applied to this product is **the Silica-(spin)Column based extraction method**

Both manual and automated method can be used. We recommend use of automated method

Extraction kit components

	
Spin column	Collection tube
	
Swab/Stool Lysis buffer	KSB Buffer(Lysis buffer)
	
KSW1 Buffer(1st Wash buffer)	KSW2 Buffer(2nd Wash buffer)
	
KSE Buffer(Elution buffer)	Proteinase k

Lid color	Components	1 kit(100T)
White	Swab/Stool Lysis buffer	100ml
Yellow	KSB Buffer	30 ml
Blue	KSW1 Buffer[1] (Concentrate)	30 ml
Pink	KSW2 Buffer[2] (Concentrate)	12 ml
White	KSE Buffer	15 ml
-	Proteinase k[3]	2 X 1.25 ml
-	Spin column	100 each
-	Collection tube	200 each

[1] Add 30mL of Absolute Ethanol before use.
[2] Add 48mL of Absolute Ethanol before use.
[3] Should be stored at 2 ~ 8℃ after reception.

Performance data

Comparison of recovery

We conducted a comparison test of RADI PREP and Qiagen recommended for use by CDC.

RNA Extraction Options

For each of the kits listed below, CDC has confirmed that the external lysis buffer is effective for inactivation of SARS-CoV-2.

Instrument/Manufacturer	Extraction Kit	Catalog No.
QIAGEN	QIAmp DSP Viral RNA Mini Kit	50 extractions (61904)
	QIAamp Viral RNA Mini Kit	50 extractions (52904) 250 extractions (52906)

Material of test

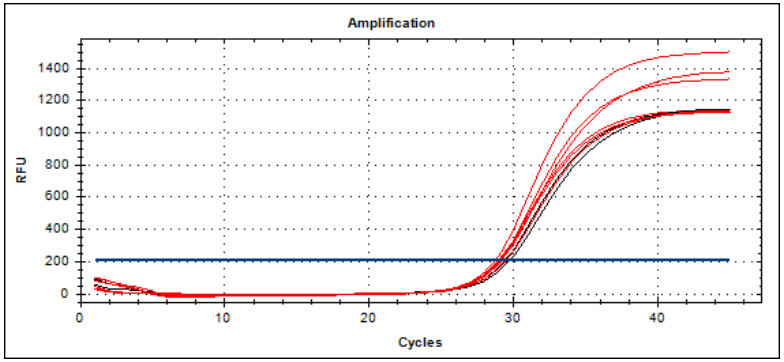
- Specimen : Negative Nasopharyngeal swab, Synthetic COVID19 RNA(100 copies/ul)
- Prep Kit : RADI PREP Swab and Stool DNA/RNA KIT(MP002), QIAamp Viral RNA mini Kit
- PCR Kit : RADI COVID-19 Detection KIT(RV008)
- Instrument : Table top Centrifuge, CFX96

Method of test

- Specimen : Follow instruction for use of RADI PREP Swab and Stool DNA/RNA KIT and QIAamp Viral RNA Mini Kit

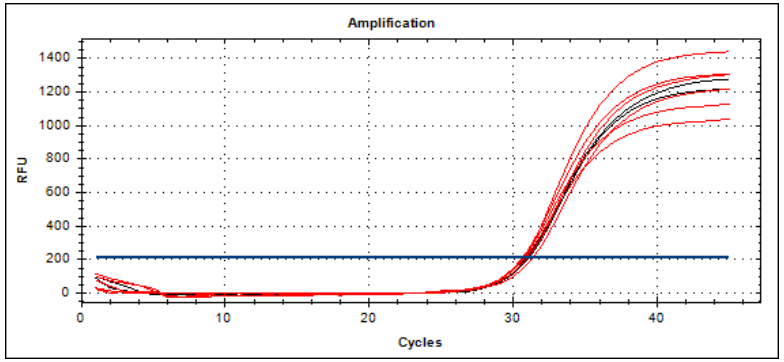
Performance data

1000 copies/rxn



Kit name	Ct value	Kit name	Ct value
RADI PREP	29.02	QIAamp	29.8
	29.17		29.44
	29.52	Average	**29.62**
	29.1	CV(%)	0.86
	29.22		
	28.79		
Average	**29.14**		
CV(%)	0.83		

500 copies/rxn

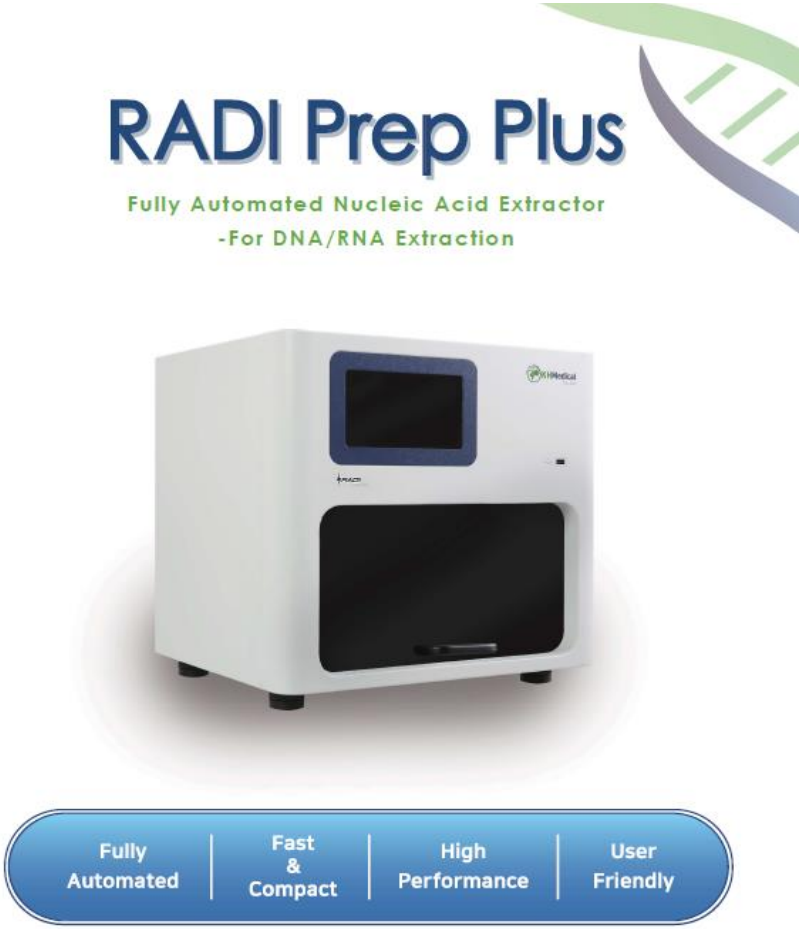


Kit name	Ct value	Kit name	Ct value
RADI PREP	30.84	QIAamp	30.95
	30.74		31.13
	31.36	Average	**31.04**
	31.04	CV(%)	0.41
	30.64		
	30.75		
Average	**30.90**		
CV(%)	0.86		

Conclusion

- RADI PREP and QIAamp have equivalent performance.

Fully Automated Extractor



- Extraction time: 15 Minutes
- Weight :18 Kg
- Battery Charged
- Fully Automated Extractor
- Samples : Swab , Stool, Blood ,dried bloo d spots etc
- Thru put : 8 Samples (One hour: 24~32 s amples)
- Cost effectiveness

New Product – POCT PCR (Including Extraction and PCR)

2. POCT (Point of Care Test) PCR

D. POCT PCR Machine & Kits

- Spec : Only 4 Kg
- Time to result: 40 minutes
- Easy extraction and PCR
- 16 Samples per run
- Target Gene : E gene and S gene for COVID-19
- Launching soon !

Future Developing Reagents

- Malaria Detection KITs
- HPV Screening KIT
- MTB KIT
- Dengue Detection KIT
- Influenza Detection KIT
- Coronavirus Detection KIT
- Food bone Screening KIT
- Fever illness Detection KIT
- Coronavirus Detection KIT

THANK YOU