ROJETECHNOLOGIES

Viga SARS COV-2 Molecular Diagnostic Kit

Store at -20 ºC in darkness

100rxn

Cat NO: MD983053

By ROJE

Edition: 02/2022

Description

Viga SARS COV-2 Molecular Diagnostic Kit is used for the qualitative diagnostic of SARS COV-2 from samples of Nasopharyngeal swab, throat swab, anterior nose swab, nasal wash, and nasal aspiration with suspected COVID-19 that are provided by health care professional staffs. This kit is designed to diagnose RdRP genes and N genes from the SARS-COV-2 genome qualitatively.

Kit content

Components	100 preps
Q-ROMAX, 4X	500µl
Pro I Mix	400µl
RTase, Recombinant Reverse Transcriptase, RNase H-	100µl
Positive Control	150µl
Negative Control	150µl

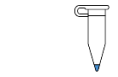
Specimen collection

Consider all samples potentially infectious and transfer them by precisely following the biosafety guidelines. The collection swab should have a synthetic tip, such as nylon or Dacron, and an aluminum or plastic shaft. A cotton swab with a wooden shaft is not recommended. After sample collection, swabs should be stored at VTM (virus transfer medium) immediately.

Specimen isolation

For viral nucleic acid isolation, use RNJia Virus Kit (REF: RN983072) or other kits approved by the health ministry.

Step 1:
Isolated nucleic acid



Step 2:
Add molecular biology grade water

Step 3:
Add Q-ROMAX, 4

Step 4
Add pro I mix

Step 5:
Add RTase enzyme

Step 6:
Add isolated nucleic acid

Step 7:
Run the PCR program

Step 8:
Interpret the PCR results

Results	Cq HEX (IC)	Cq TexasRed (N)	Cq FAM	
A	+/-	Ct<40	Ct<40	Covid 19 positive
A	+/-	Ct<40	-	Covid19 positive
A	+/-	-	Ct<40	Covid19 positive
R	-	-	-	False Negative
A	-	-	-	Negative Control
A	Ct<40	-	-	Negative clinical sample

Process

Take out each component from the kit and place them on the benchtop. Allow the reagents to equilibrate to room temperature, then briefly vortex each tube for later use. The volume of the isolated sample in this test should be 10µl. Prepare PCR reaction and then perform Real-time PCR according to Table 1 and Table 2, respectively.

Table 1: PCR reaction preparation

Components	Volume
Q-ROMAX, 4X	5µl
Pro I Mix	4µl
RTase, Recombinant Reverse Transcriptase, RNase H-	1µl
Isolated RNA	10µl

Table 2: one-step Multiple Real Time-RT-PCR

Cycles#	Temp	Time	Stage
1	50ºC	20 min	cDNA synthesis
1	95ºC	3 min	Polymerase activation
45 cycles	95ºC	10s	Denaturation
	60ºC	40s	Annealing and extension of nucleic acid and measurement of fluorescence in green, yellow, and orange channels

Interpretation of results

- Data analysis for each gene should be done separately by using a manual threshold. The threshold for each sample should be considered in the curved exponential phase of fluorescence and upper than background signal.

Green FAM fluorophore for RdRp gene, orange Texas Red fluorophore for N gene, and yellow HEX fluorophore for RNase P (internal control).

- NTC is used for controlling contamination. Increasing the amount of fluorescence curve in NTC does not pass the threshold. Ct lowers than 35 for NTC could be PCR artifacts (Atypical shape of S curve).
- Internal control or RNase P gene Ct should be 40 or lower than it in all clinical samples. This shows the presence of human nucleic acid and also the acceptable quality of the sample.
- RNase P Ct>40 or no observed Ct indicate low sample concentration or PCR inhibitor. In this condition, it is recommended to dilute the isolated sample 1:2 and repeat the PCR test. If the result is not acceptable again, isolate another fresh sample from a patient and repeat the test.
- The positive clinical sample must have Ct≤40.
- If a positive reaction does not show a typical S-shaped curve, the performed test is not acceptable. So, repeat the test by following the instructions.
- Determine the cause of defects in positive control reaction. Do corrective actions and document them.
- For results interpretation about positive and negative controls, refer to Table 3.

Table 3: Acceptable situation for positive and negative control (NTC)

Results	Ct HEX (IC)	Ct Texas Red (N)	Ct FAM	
A	+/-	Ct<40	Ct<40	**Covid19 positive**
A	+/-	Ct<40	-	**Covid19 positive**
A	+/-	-	Ct<40	**Covid19 positive**
R	-	-	-	**False Negative**

ROJETECHNOLOGIES

A	-	-	-	**Negative Control**
A	Ct<40	-	-	**Negative clinical sample**

Result of (-): Ct value >40 or undetermined, Result of (+): Ct value ≤ 40*

*NTC: NTC is used for controlling contamination. Increasing the amount of fluorescence curve in NTC does not pass the threshold.

Limitations

- Low virus titration in a patient sample, improper sample transportation, and low template quality lead to a false-negative result.
- Coronavirus genome genetic diversity can lead to false-negative results.
- If control results are not valid, other reactions cannot be interpreted. All positive results must have S-shaped curves.
- The Minimum limit of detection is 100 copies/ml.