Viga SARS COV-2 Molecular Diagnostic Kit

Store at -20 ºC in darkness

100rxn

Cat NO: MD983053

By ROJE

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Description

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

Kit content

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

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. cotton swab with wooden shafts 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 with confirmation of ministry health.

Process

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

Table 1: PCR reaction preparation

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

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
	55ºC	45 s	Annealing and extension
	72ºC	15 s	Final extension

Interpretation of results

- Data analysis for each gene should be done separately by using manual threshold. Threshold for each sample should be consider in 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, is considered as possible contamination. In case of strong signals, the Ct upper than 35 for NTC could be PCR artifacts (A typical 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 acceptable quality of 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 patient and repeat the test.
- The positive clinical sample must have Ct≤40.
- If positive reaction does not show typical S-shaped curve, the performed test is not acceptable. So, repeat the test again by following the instruction.
- 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

Situation	IC HEX	N Texas Red	RdRp FAM
Positive control	N.A	+	+
		–	+
		+	–
Negative control	+	–	–
Not accepted results	–	–	–

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

Limitations

- Low virus titration in patient sample, improper sample transportation and low quality of template, lead to 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.
- Minimum limit of detection is 100 copy/ml.