# Viga SARS COV-2 and Influenza A/B Molecular Diagnostic Kit

Store at -20 ºC in darkness

100 rxn

Cat NO: MD983007

**By ROJE**

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## Kit content

| Components | 100 Preps |
|---|---|
| Q-ROMAX, 4X | 500 µl |
| Pro II Mix | 400 µl |
| RTase, Recombinant Reverse Transcriptase, RNase H- | 100 µl |
| Positive Control | 150 µl |
| Negative control | 150 µl |

## Description

The Viga SARS COV-2 and Influenza A/B Molecular Diagnostic Kit is based on one step RT-PCR reverse transcriptase reaction. A part of the RNA sequence of Pathogen is converted to cDNA and then used as a PCR reaction template. The resulting PCR product is identified by an oligonucleotide probe labeled with fluorescent color. This Kit detects *N* gene from SARS COV-2 as well as *M2* gene of the Influenza A virus, and *NS1* gene of Influenza B virus. Other Coronaviruses and other strains of Influenza virus are not identified with this Kit.

## 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 (REF: RN983072), RNJia Virus Kit or other kits approved by the ministry of 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.

**Table1:** PCR reaction preparation

| Components | volume |
|---|---|
| Q-ROMAX, 4X | µl 5 |
| RTase, Recombinant Reverse Transcriptase | µl 1 |
| Pro II Mix | µl 4 |
| Isolated RNA | µl 10 |

**Table 2:** One-step multiple Real time RT-PCR

| Cycle # | Temp | time | Stage |
|---|---|---|---|
| 1 | 50°C | 20min | cDNA synthesis |
| 1 | 95°C | 3min | Polymerase activation |
| 45 | 95°C | 10min | Denaturation |
| | 55°C | 45min | Binding and amplification of nucleic acid |
| | 72°C | 15min | Final amplification |

**Interpretation of results**

- To analyze the PCR results, select FAM channel for Influenza A, Yakima Yellow channel for Influenza B virus, Texas Red channel for SARS CoV-2 and cy5 channel for RNase P gene.
- Please check both amplification curves and Cq for each sample. The linear and logarithmic diagrams of the sample should both be checked and compared to the negative control.
- Evaluation of results should be done after reviewing positive and negative controls and confirming their acceptance. If control result is not acceptable, the patient's result cannot be interpreted.
- Changes in Cq values in positive control may indicate partial inhibition of PCR.

**Table 3:** Valid control criteria

| Results | Cq Cy5 | Cq Texas Red | Cq Yakima | Cq FAM | |
|---|---|---|---|---|---|
| A | - | Ct> 40 | Ct> 40 | Ct> 40 | Influ A/B**, COVID-19 |
| R | - | - | - | - | False negative |
| A | - | - | - | Ct> 40 | Influ A + |
| A | - | - | Ct> 40 | - | Influ B+ |
| A | - | Ct> 40 | - | - | COVID-19+ |
| A | - | - | - | - | NTC |
| A | 35 - 40 | - | - | - | Negative control |

*A: Accept, R: Reject

**Influ A/B: Influenza A and B

**limitation**

- The optimal performance of this test also depends on how samples are collected, transferred and stored.
- This kit is suitable for diagnosing target viruses in swab samples and respiratory sputum. A negative test does not reject the possibility of SARS CoV-2, Influenza A or B virus, because test result may be affected by sample collection, user error, how the sample is mixed or low virus titration that can be less than the sensitivity of the Kit.
- The presence of PCR inhibitors can cause false negative results.
- Sequence diversity in the target area of unknown types of viruses may lead to false negative results or less Kit sensitivity. In these cases, the results should be interpreted based on clinical findings and other tests.
- The limit of detection in Viga SARS CoV-2 and Influenza A/B Molecular Diagnostic Kit is 200 copies per ml for COVID-19 and 150 copies per ml for Influenza A/B, respectively.