ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test intended for qualitative detection of nucleic acid from the SARS-CoV-2 in oropharyngeal/nasopharyngeal swabs and saliva specimens.

Results are the identification of SARS-CoV-2 RNA. The RNA is generally detective in oropharyngeal/nasopharyngeal swab and saliva specimens during infection. Positive results are indicative of active infection with SARS-CoV-2.

The test consists of three processes in a single tube assay:
• Reverse transcription of target RNA and Internal Control RNA to cDNA
• PCR amplification of target and Internal Control cDNA
• Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The diagnostis kit contains:

- RT-PCR Reaction Mix (850 µl/tube) composed of 3 set of primers and probe designed to detect the Sars Cov 2 RNA:
  - **ORF1 ab** that is detected with *FAM* fluorophore
  - **E gene** that is detected by *VIC* fluorophore
  - **N gene** that is detected by *ROX* fluorophore.

  and 1 set of primers and probes designed to detect the internal control:
  - **Rnase-P** that is labeled with *Cy5* fluorophore.
- Enzyme mix (150 µl/tube) composed of Taq polymerase, Reverse transcriptase and UDG (Uracil DNA glycosylase)
- Positive control (1000 µl/tube)
- Negative control (1000 µl/tube)

ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit shall be stored at -25°C to -15°C. It's important to protect SARS-CoV-2 Assay which contains fluorogenic probes from light.

Reagent preparation

All the reagents and controls need to be equilibrated at room temperature, exept for the enzyme. The Enzyme Mix has to be kept in cooler or on ice during the preparation and use. All the reagents and controls are vortexed for 10 seconds, centrifuged briefly to collect the content to the bottom of the tube.

Preparation of RT-qPCR Mix according to the formula described in the Table below:

Reagent Name	Volume in µL per Reaction	Volume in µL per N Reactions
RT-qPCR Reaction Mix	8.5	8.5 × (N+1)
Enzyme Mix	1.5	1.5 × (N+1)
Total Volume	10	10 × (N+1)

After the mix is prepared, add **10µL of RT-qPCR Mix** into each required well of an appropriate optical 96-well reaction plate and **10µL of extracted RNA** of samples, Positive Control and Negative Control into each well. Seal the optical 96-well reaction plate with optical adhesive file, spin the optical 96-well reaction plate in order to avoid drops on the foil and collect the entire content to the bottom of the wells.

RT-qPCR amplification

In Biorad CFX-96 the general setting to define are:

- Sample Volume: 20µL
- The Fluorescent Detectors (Dye) that are four (FAM, VIC, ROX, CY5)
- The Thermal Cycle profile: "File-Open-New protocol"

Stage	**Temperature**	**Time**	**Cycle number**
1	50°	2 minutes	1
2	95°	2 seconds	1
3	95°	1 second	41
	60°	13 second	

Fluorescent signal is collected at 60℃ step.

Analyzing the data using Biorad CFX-96 software:

- Select FAM, VIC, ROX and CY5 to setting the Baseline Threshold, respectively. Unselect the box for "Auto Calculated".
- For Single threshold setting: manually drag the threshold line until it lies within the exponential phase of the fluorescence curve and above any background signal.
- Determine the cycle threshold (Ct) values for each assay.

Interpretation of results

Speciment ID	ORF1ab (FAM)	N gene (ROX)	E gene (VIC)	Internal Control (Cy5)	Results	Interpreration
A	+	+	+	+/-	SARS-CoV2 positive	Sars-cov 2 RNA is detected
B	+	-	+/-	+/-	SARS-CoV2 positive	Sars-cov 2 RNA is detected
C	-	+	+/-	+/-		
D	+	+	+/-	+/-		
E	-	-	+	+/-	Presumptive positive for SAR-COV2	Sarbecovirus RNA is detected but not specific SARS-COV2
F	-	-	-	+	SARS-CoV2 negative	Sars-cov 2 RNA is not detected
G	-	-	-	-	Invalid	Repeat the test

"+" means "Detected", and the Ct value ≤ 40. And "- "means "Not detected", and the Ct value >40.

E gene could not distinguish between SARS-CoV-2 and SARS-CoV. Additional confirmatory testing may be conducted if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV or other Sarbecovirus currently unknown to infect humans.

The analytical sensitivity of this kit is 200 copies/ml. The kit is reliable because is possible in one step detect N, E and ORF1ab genes of SARS-CoV-2 RNA that indicate the current infection and these results are obtained in a short time (around 40 minutes).

This kit meets safety, quality and performance requirements.