110 / 220 / 330 tests (Ready to use kit)

The SARS CoV-2 is a new virus that belongs to the coronavirus family. It is formed by a positive single stranded RNA. It can cause acute respiratory disease and severe pneumonia in humans. The SARS CoV-2 virus sequence shows similarities with the beta-coronaviruses found in bats, but they are genetically distinct from other coronaviruses such as SARS CoV and MERS-CoV although they have similarities that can cause Severe Acute Respiratory Syndrome and even death. Today there are several reported sequences of this virus and our kits are designed to detect them all.
OnePCR-realtime™ SARS CoV-2 RNA Real Time PCR Kit is a screening assay for rapid and accurate detection of SARS CoV-2.

Principles of the test:

Multiplex One Step Bioingentech® RT-qPCR Kits provide components for onestep reverse transcription and quantitative PCR (RT-qPCR) in a convenient format that is compatible with both rapid and standard qPCR cycling conditions. Both cDNA synthesis and PCR are performed in a single tube using gene specific primers and either total RNA or mRNA. These one step RT-qPCR kits have been formulated for use with fluorogenic probe-based 5' nuclease technology probes.
The One qPCR Enzyme Mix includes Reverse Transcriptase, Recombinant Ribonuclease Inhibitor in an optimized formulation, also include Bioingentech® Taq DNA polymerase and all reagents for an optimized RT-qPCR. The SARS CoV-2 (E-ORF1ab-S gene) primer and probe mix provided in the kit hybridize in specific and conserved zones of two genes. The first one is E gene which generate an envelope protein which plays a central role in virus morphogenesis and assembly, ORF1ab which generates a replicase polyprotein involved in the virus transcription and replication, on the other hand, S gene generate a Spyke glycoprotein on the virion surface which mediates receptor recognition and membrane fusion. These genes can be detected through your real time platform by the 5' nuclease PCR detection method. During PCR amplification, forward and reverse primers hybridize to the SARS CoV-2 target cDNA generated. Fluorogenic probes are included in the same reaction mixture. E gene is amplified and detected in ROX channel; ORF1ab gene is amplified and detected in FAM channel and S gene is amplified and detected in CY5. An additional primer/probe set to detect the human RNase P gene (RP) as internal control to monitor PCR inhibition is also included in the panel labeled with a 5-reporter and a 3-quencher **(see Table 8 and 9)**. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. As a result, a fluorescence increase can be detected on a range of real time PCR platforms through HEX/VIC channel.

Our kits also include Positive and Negative Control which are detailed in FAQ section.

Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect SARS CoV-2 in real time PCR. its allows relative/absolute quantification as well as qualitative assay.
Real time PCR is based on fluorogenic dyes. Ct value between 12- 36 should be taken positive. Values between 36-40 Ct should be taken as marginal Positive. Ct above 40 must be considered as negative **(see table 5).**

This kit need RNA as a template which can be isolated from nasopharynges swab, sputum, oropharynges swab and others. We have detail procedures available for RNA extraction from these samples, using the Trizol method.
We do not recomend the use of affinity columns due to reports that indicating purification problems arising from the lipids present in the biological sample, which quickly clog the column decreasing its performance.

Table 1. Kit Components:

Reactions Tubes
Universal qPCR Master Mix
Primer, Probe and Internal Control Mix
One RT-qPCR Enzyme Mix
SARS CoV-2 (E-ORF1ab-S Genes) Positive Control
SARS CoV-2 (E-ORF1ab-S Genes) Negative Control
PCR grade Water

Table 2. Instrument Compatibility:

* ABI 7300	* LightCycler 2.0
* ABI 7500FAST	* LightCycler 480
* ABI 7900	* Mastercycler® ep realplex
* AB Step One	* Mx3000P QPCR System
* AB Step One Plus	* Mx3005P QPCR System
* Agilent Mx3005P	* RotorGene 3000
* CFX96 & CFX384	* RotorGene 6000
* ExiCyclerTM 96	* RotorGeneQ
* iQ5 & MyiQ Cycler	* SLAN® Real-Time PCR
* Illumina Eco	* Smartcycles II
* LightCycler Nano	* QuantGene 9600
* CFX Opus	* QuantStudio™ 3-5-6-7

Procedure:

Please read through the entire procedure before starting.

Before Starting

- Pulse-spin each tube in a centrifuge before opening.
- Homogenize the solutions for 5 seconds prior to pipetting.
- You must consider use different tips in order to avoid cross contamination.
- Use only sterile, RNAses, DNAases and pyrogens free tips.

Step 1

Prepare a Master mix according to the reaction table 3.

Table 3. Reaction components for PCR

Reaction Tubes	Sample and Internal Control	Positive Control	Negative Control
Universal qPCR Master Mix	7,5 µL	7,5 µL	7,5 µL
Primer, Probes and Internal Control Mix	0,3 µL	0,3 µL	0,3 µL
One qRT-PCR Enzime Mix	0,3 µL	0,3 µL	0,3 µL
PCR grade water	3,4 µL	3,4 µL	3,4 µL
RNA Sample	3,5 µL		
SARS CoV-2 (E-ORF1ab-S genes) Positive Control		3,5 µL	
SARS CoV-2 (E-ORF1ab-S genes) Negative Control			3,5 µL
Total Volume	15 µL	15 µL	15 µL

Step 2

Place the tube in a cycler and perform One Step qPCR according to the program outlined in Table 4.

Table 4. Six Step PCR Cycling table

Cycle	Steps	Time	Temp.(°C)
1 Cycle	Activation Step	2 min	25°C
1 Cycle	Reverse Transcription	15 min	50°C
1 Cycle	Denaturation	2 min	95°C
40 Cycles	Annealing	3 seg	95°C
	Extension	30 seg	55°C

Interpretation of the test

1) Qualitative analysis:

Ct (Threshold cycle) value of each sample can be read as follows.

Table 5. Ct value result

Ct value	Result
0 - 11	Negative
12 - 36	Positive
36 - 40	Marginal Positive
> 40	Negative

* Is important to mention that Ct value over 40 are considered as Negative results. If Ct value is in between a 12 - 36 range, it must be considered as Positive result. This depends of the sample initial concentration used in each reaction. You should consider that sample real concentration could be modify by the sample purity when this is quantified.

* For more technical information you must request the quality control for each kits. Also you can request more information writing to our email: info@bioingentech.com

2) Quantitative analysis:

Table 6. Preparation of standard curve dilution series. SARS CoV-2 positive control:

Average Positive Control Concentration	
SARS CoV-2 (E-ORF1ab-S Genes)	See Quality Control

Standar curve	Preparation series a fresh dilution	Concentration	Copy Number
Tube N°1:	2 µl SARS CoV-2 (E-ORF1ab-S Genes) Control (0,1 ng/µL) + 18 µL de PCR grade Water	See quality control	See quality control
Tube N°2:	2 µl Tube N°1 + 18 µL PCR grade Water	See quality control	See quality control
Tube N°3:	2 µl Tube N°2 + 18 µL PCR grade Water	See quality control	See quality control
Tube N°4:	2 µl Tube N°3 + 18 µL PCR grade Water	See quality control	See quality control
Tube N°5:	2 µl Tube N°4 + 18 µL PCR grade Water	See quality control	See quality control
Tube N°6:	2 µl Tube N°5 + 18 µL PCR grade Water	See quality control	See quality control
Tube N°7	2 µl Tube N°6 + 18 µL PCR grade Water	See quality control	See quality control

* Important Note: Don't forget Homogenize the tubes.

* We will send a Quality Control report for each purchase.
** For reaction mix you must use Universal qPCR Master Mix.
*** If you want to obtain less DNA copies you must include a new dilution tube (Tube N° 8). Note: Final DNA copy number will depend of the DNA concentration (you can see it in Quality Control Report).

Table 7. Standard curve set up

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F	Tube G
Universal qPCR Master Mix	7,5 µL	7,5 µL	7,5 µL	7,5 µL	7,5 µL	7,5 µL	7,5 µL
Primer, Probes and Internal Control Mix	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL
Enzyme Mix	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL	0,3 µL
PCR Grade Water	3,4 µL	3,4 µL	3,4 µL	3,4 µL	3,4 µL	3,4 µL	3,4 µL
Tube N° 1 (Positive Control)	2 µL						
Tube N° 2		2 µL					
Tube N° 3			2 µL				
Tube N° 4				2 µL			
Tube N° 5					2 µL		
Tube N° 6						2 µL	
Tube N° 7							2 µL
Total Volume	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL

2.1.-Assess the Ct value when amplification curve of Standard tube 1, 2, 3, 4, 5, 6 passes the threshold line. However, four tubes are sufficient for standard curve. (tube1-tube4).
2.2.- Calculate quantitative value to compare with Ct value of unknown samples and curve of Standard tube 1, 2, 3, 4, 5, 6.

3) Test validation:

3.1.- Each Ct value standard should be as follows.
Standard 1 < Standard 2 < Standard 3 < Standard 4 < Standard 5 < Standard 6.
3.2.- R-value of standard curve should be 0.900 -0.999.
R-Value represent how well the experimental data fit the regression line. A significant difference in observed Ct values between replicates will lower the R-value.
3.3.- The standard curve slope result should be all negative.
3.4.- The desired amplification efficiences vary from 90% to 110%. The theorical maximum of 100% indicates that the polymerase enzyme is functioning at its maximum capacity.
Low reaction efficiences may be caused by poor primer design or by suboptimal reaction conditions. Reaction efficiences > 110 may indicate pipetting error in your serial dilutions or coamplification of nonspecific products, such as primer-dimers.

Visual explanation FAQ:

1.-Positive control:

The Positive control assay uses a ROX/FAM/CY5 dye and should be detected through the Orange/Green/Red channel of your real time PCR instrument.
For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of SARS CoV-2 (E-ORF1ab-S) genes copy number / Ct value.
Alternatively, the positive control can be used at a single dilution SARS CoV-2 (E-ORF1ab-S) genes where full quantitative analysis of the sample is not required. Each time the kit is used, at least one positive control reaction must be included in the run.
Particularly, due to amount of this reagent, you should run a positive control for each 12 samples. A positive result indicates that the primer and probes for detecting the target SARS CoV-2 (E-ORF1ab-S) genes worked properly in that particular experimental scenario. If a negative result is obtained the test results should be invalid and must be repeated **(see Table 11)**. Seal all other samples and negative controls before pipetting the positive control into the positive control well tube.

2.-Internal Control:

The internal control is included in Primer, Probe and Internal Control Mix along to the target pathogen detection. In order to interpreted results, read the yellow channel. The internal control assay uses a HEX dye and should be detected through the Yellow channel of your real time PCR instrumenet and give a Ct value of 28 (+/- 5) depending on the level of sample dilution and concentration. A positive result through the Yellow channel therefore indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the Yellow channel the result should be analyzed by combination of result, follow the **Table 11** data.

3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. **Particulary, due to amount of this reagent, you should run a negative control for each 12 samples.** In this instance the SARS CoV-2 (E-ORF1ab-S) genes Negative Control should be used in place of template. A negative result indicate that the reagents have not become contaminated. If a positive result and Ct value less than 36 is obtained, the results should be analyzed and check if a correct amplification curve was obtained. Whe you obtain a clear amplification curve you should consider repeat your assay due to probably the sample was contimanated **(see table 11).**

***Remember: Run a positive control and negative control for each 12 samples. For reaction mix you must use Universal qPCR Master Mix.**

Table 8. Fluorogenic probes, Channels and Dyes

Channel	Source	Detector	Dyes
Green	470 (Nm)	520 (Nm)	FAM, Sybr green1, Fluorescein, Eva green, Alerxa fluor 488.
Yellow	530 (Nm)	550 (Nm)	Joe, Vic, HEX, Tet, Cal Fluorgold 540, YaKima Yellow.
Orange	585 (Nm)	610 (Nm)	ROX, Cal Fluor Red 610, Cy3, Texas Red, Alexa Fluor 568.
Red	625 (Nm)	660 (Nm)	Cy5, Quasar 670, Lightcycler, Red 640, Alexa Fluor 633.
Crimson	680 (Nm)	710 (Nm)	Quasar 705, Lightcycler Red 705, Alexa Fluor 680.

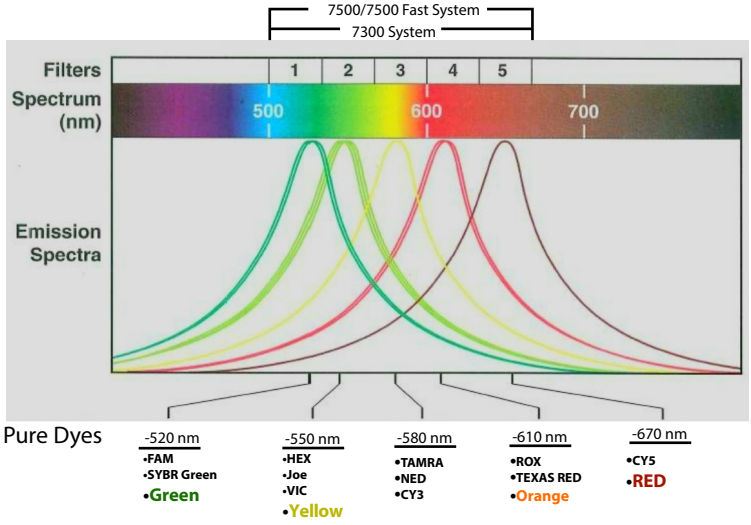

Table 9. Fluorophores Table.

	Channel	Dyes
1.- Positive Control	Orange/Green/Red	ROX/FAM/CY5
2.- Internal Control	Yellow	HEX
3.- Sample Target E	Orange	ROX
4.-Sample Target ORF1ab	Green	FAM
5.-Sample Target S	Red	CY5

Important Note:

Probes for sample and controls mentioned in manuals are just a reference and it does not imply that these probes will be the final fluorophores for a purchased kit. The probe combination will be depending of several factor and this information will be available in the Certificate of analysis when you purchase one of our PCR Kits.

Is important to mention that we can develop special request for other pathogens or multiplex detection according client's specifications

We strongly recommend don't use or combine our products with reagents from another kits or unknown provenance. We can't assure good result if incompatibility problems occur.

Temperature

All our reagents are made through protein engineering and are stable at room temperature, the label temperature is just a recommendation after the product is open.

Table 10. Store Temperature Kits

Store temperature	The label temperature
Shipping temperature	At room temperature

Table 11. Interpretation of Results

Sample	I.C	N.C	P.C	
+	+	-	+	POSITIVE
+	-	-	+	POSITIVE
+	+	+	+	Check Ct and Consider repeat assay*
+	-	+	+	Check Ct and Consider repeat assay*
+	+	-	-	Check Ct and Consider repeat assay*
+	+	+	-	Check Ct and Consider repeat assay*
+	+	-	-	Exp. Fail
+	+	+	-	Exp. Fail
+	-	-	-	Exp. Fail
+	-	+	-	Exp. Fail
-	+ / -	+ / -	+ / -	NEGATIVE

The result is considerer positive if two of the tree genes detected is positive.

* Sometimes amplification curves for Negative or Internal control with CT< 30 is generated, but its not necessary a Positive result. You should see and determinate if is a sigmoid curve. If the amplification curve isnt sigmoid you should considere as negative result.

Table 12. Products Specifications

Technology	5' nuclease probe based real time PCR assay.
Type of nucleic acid Kit	RNA
Kit storage	Shipped at room temperature, the label temperature is just a recomendation after the product is open.
Detection Limit	See Quality Control
Sensitivity & Specify	See Quality Control
Controls included	Positive Control and Negative Control.
Channels	ROX Orange Channel detect E gene amplification FAM Green channel detect ORF1ab gene amplification CY5 Red Channel detect S gene amplification HEX Yellow Channel detect internal Control.