INSTRUCTION FOR USE
Real-Time PCR reagents kit – YZZY Test Covid-19

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INTENDED USE

The YZZY test COVID-19 Kit is intended for the qualitative detection of SARS-CoV-2 virus RNA by one-step RT-PCR assay with Real-Time detection.
The YZZY test COVID-19 Kit is intended for use by qualified clinical laboratory personnel that was specifically instructed and trained on the real-time RT-PCR assays techniques.

SUMMARY AND EXPLANATION OF THE TEST

Nucleic acid extraction and purification is to perform using the YZZY RNA Magnetic Kit manual procedure (automatic extraction assay protocol is optional) or any other extraction and purification kit intended for RT-PCR ASSAY with Real-Time detection. The YZZY test COVID-19 Kit was validated for the following Real-Time PCR instruments: Mx 3005P™ QPCR System (Agilent), Rotor-Gene® 6000 (Corbett Research), Rotor-Gene® Q5/6 plex Platform (QIAGEN), CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad) • CFX96™ Deep Well Dx System (Bio-Rad), • CFX96™ Real-Time PCR Detection System (Bio-Rad), • CFX96™ Dx System (Bio-Rad), CFX96 Touch Real-Time PCR Detection System (Bio-Rad).
The YZZY test COVID-19 Kit primers and probes sets are designed to detect RNA from the SARS COV-2 **N gene** and **RdRP gene**.
The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.
The system contains sufficient reagents for 100 RT-PCR reactions. The sensitivity of the system is 20 copies of SARS-CoV-2 RNA per reaction. The YZZY test COVID-19 Kit cross-reactivity with other respiratory viruses was tested by using in-silico BLAST analysis.

DESCRIPTION

The YZZY test COVID-19 Kit detects two SARS-CoV-2 specific target sequences from the Nucleocapsid gene (N) and RdRP gene (Target 1 and Target 2 - HEX/Yellow channel) of the virus and Exogenous Internal control - FAM/Green channel. The primers and probes used in the RT-PCR assay are based on the WHO and CDC published primer and probe sequences. In addition to the Internal control, the test uses both Negative Extraction Control (NEC control which is taken through all procedural steps, including the extraction), and Positive Control that monitors integrity of reagents and testing procedure accuracy.

REAGENTS AND MATERIALS

Materials Provided

The content of the YZZY test COVID-19 Kit is indicated in Chart 1.

Chart 1. The content of the YZZY test COVID-19 Kit.

Component	Qu-ty / Volume
1. RT-PCR reagent	1 tube – 0.5 ml
2. Primers COVID-19	1 tube - 1.0 ml
3. Positive Control (PC-R)	1 tube - 0.2 ml
4. Negative control (NC)	1 tube - 1.0 ml
5. Internal control (IC-R)	1 tube – 1.0 ml

Reagent Storage and Handling

Transportation conditions of the Kit are:
- under ambient temperature, but not higher than 25 ° C (up to 10 days);
- under + 2-8 °C temperature up to 30 days.

Delivery by any means of transport is allowed under conditions ensuring safety, in accordance with the rules for the carriage of goods operating on this type of transport.
Storage condition of the YZZY test COVID-19 Kit is -20 °C temperature.
It is allowed to freeze/thaw the components of the test system up to 10 times.
The shelf life of the YZZY test COVID-19 Kit is 12 months from the manufacturing date.

EQUIPMENT AND MATERIALS REQUIRED (not provided)

- Pipettes (0.5 µl – 1000 µl);
- Nuclease-free aerosol-resistant pipette tips with filters;
- Sterile 1.5 ml tubes;
- Benchtop centrifuge with angle rotor 1.5 – 2.0 ml tubes (RCF ≥ 10,000 × g);
- Vortex mixer;
- Disposable gloves, powderless;
- Biohazard waste container;
- Refrigerator and freezer;
- Tube racks;

- Real-time PCR Analyzer (e.g. Qiagen Rotor-Gene Q; Bio-Rad CFX 96; Agilent MX 3005P and etc.);
- PCR tubes / striped PCR tubes with striped caps / PCR plates with Microseal, suitable for qPCR.

GENERAL LABORATORY WARNINGS AND PRECAUTIONS

This assay is for *in vitro* diagnostic use by laboratory personnel.
Responsive laboratory must comply with the relevant practises and requirements in the field of clinical PCR diagnostics.
While working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

YZZY test COVID-19 Kit SET UP

Sample Preparation and RNA Extraction

Isolation of RNA from clinical material is carried out together with IC-R (10 µl per isolation) in accordance with the instructions of the manufacturer of reagents for purification of RNA.
Important!
-Internal control contains unprotected RNA. It cannot be added directly to the sample. Add the internal control to the "Salting Out" solution of "Art RNA Extract kit" or the appropriate buffer of other RNA isolation kit which will prevent RNA degradation.
-The Negative Extraction Control (NEC) should be run through with the entire sample preparation process, from sample extraction to rRT-PCR.

RT-PCR Reaction Setup

1. Thaw all reagents if required, make gentle mix by inverting the tubes several times and centrifuge the tubes briefly to collect the components at the bottom.
2. Use compatible single PCR tube / striped PCR tubes with striped caps / PCR plates with Microseal, suitable for qPCR (read on PCR tube). If use single PCR tube / striped PCR tubes with striped caps count the right amount (**N**).
3. Use a tube 1.5 – 2.0 ml for the "Master Mix" preparation:
Add 5 x (N + 1) µl of RT-PCR reagent + 10 x (N + 1) µl of primers to a Master Mix tube.
Where **N** - is the total number of amplification reactions considering control samples. Rounding up values is allowed.

4. Mix the Master Mix by 5-fold inverting the tubes, sediment by short-term centrifugation and add **15 µl** to the prepared PCR tube.
5. Use two RT-PCR Assay's controls.
Positive Control (PC): Add 10 µl of the PC-R to the marked PCR tube.
Negative Extraction control (NEC): Add 10 µl of the NEC, to the marked PCR tube (NC which is taken through all steps, including the extraction).

6. Add 10 µl of the Samples to the prepared PCR tube.

Chart 2. RT-PCR reaction summary.

Component	Volume
Master Mix	**15**
Sample/Control	**10**
Total volume	**25**

7. Seal PCR tubes. If there are bubbles or drops on the walls of the tubes, remove by short-term centrifugation.

Instrument Setup

1. Put the PCR tubes into the reaction block of Real-Time PCR Analyzer.
2. Program RT-PCR Assay procedure in accordance with Analyzer's manual and according to chart 3.

Chart 3. RT-PCR parameters

Step	Temperature °C	Time	Number of cycles
Reverse transcription	55	10 min	1
Initial denaturation	95	15 min	1
Denaturation	95	15 sec	45
Annealing / Detection (FAM/HEX)	60	30 sec	
Elongation	67	15 sec	

3. Set positions of the Samples and controls. If it allowed by software, set it during or by the end of amplification.
4. Set Optical reporters **FAM/Green, HEX/Yellow.**
5. Set name of a run and save it. Start Run *.

* For Rotor-Gene device select the function: "Perform Calibration Before 1st Acquisition / Perform Optimization Before 1st Acquisition / Perform optimization at the 1st detection step" before starting. For all the reporters set the parameters "Min Reading / Min. Signal"- 5Fl and" Max Read-ing / Max. Signal "- 10Fl.

DATA ANALYSIS
1. Choose a logarithmic scale to reflect the results and visually check the intersection of the threshold line in the linear part of the growth of the amplification curve. If the threshold line intersects the amplification curve in a non-linear section, move it manually to the required level.
2. The results of the analysis are interpreted based on the presence/absence of the intersection of the fluorescence curve with the threshold line (which corresponds to the presence/absence of the threshold cycle value "Ct" in the corresponding column in the results table).
3. Make sure that the PCR study is valid: the control points of the analysis should correspond to the values given in chart 3.

Interpretation of Results

CT Value	Result
≤40	Detected (+)
≥40 or N/A	Not Detected (-)

Quality Control.
If the control points do not meet the required values, the analysis must be re-done, starting from the RNA extraction stage.

Chart 4. Evaluation of control point analysis results.

Checkpoint	Controlled Analysis stage	The value of "Ct" by HEX/Yellow reporter	The value of "Ct" by FAM/Green reporter
NEC	RNA extraction	–	+
PC	PCR	+	+

1. Interpret the results of the PCR analysis of the studied samples in accordance with chart 5.

Chart 5. Sample interpretation.

Sample result	The value of "Ct" by HEX/Yellow reporter (Virus RNA)	The value of "Ct" byFAM/Green reporter (Internal Control)
Positive	+	+/-
Negative	–	+
Invalide	–	–

- Samples with or without signal of Internal Control (FAM reporter) and Ct < 40 for the virus targets (HEX reporter) will be classified as SARS-CoV-2 Positive.
- Samples with a Ct < 40 for the Internal Control (FAM reporter) target and no amplification of the virus targets (No signal or Ct≥ 40 HEX reporter) will be classified as SARS-CoV-2 Negative.
- Samples with a Ct value (FAM reporter) for the Internal Control target ≥ 40 and no amplification of the virus targets (HEX reporter) are considered invalid. The samples must be re-extracted and re-tested. If the problem persists, a new sample must be obtained.

Important! Visually inspect one-by-one the amplification curve for each sample that exhibitsSARS-CoV-2 signal. It is important to inspect each sample with SARS-CoV-2 signal individually. The presence of signal from other samples may automatically adjust the scale of the Y axis to a higher RFU making it impossible to analyze the curve of samples with late and/or weak SARS-CoV-2 signal in detail.

TROUBLESHOOTING

Problem	Possible cause	Solutions
No signal on FAM/Green channel	Using inappropriate consumables	Use consumables suitable for qPCR compatible with Real-Time PCR Analyzer
	PCR inhibition	Dilute Sample 5 times, use RNAse free water, repeat the analysis
		Use a new specimen of the patient
		Make RNA extraction and isolation using kit from another manufacturer
No signal in PC	Incorrect preparation of PCR-mix	Carefully prepare a new PCR mix
	Incorrect amplification parameters	Set amplification settings according to Chart 3
	Bad storage conditions (outdated reagents)	Use new Kits that were stored under the right conditions
The presence of a signal on HEX / Yellow porter in NC and /or NTC	Contamination	- Perform decontamination procedures; - Use a new Kit

NOTES:

1 By agreement with the buyer, it is allowed to change the configuration of the Kit that does not affect the key points of the analysis.

AUTHORIZED BY
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