INSTRUCTION FOR USE
YZZY Test Covid-19 Express Kit
(With endogenous Internal control)
Table of Contents

INTRODUCTION

Intended Use

The YZZY test Covid-19 Express Kit intended for the FAST qualitative detection of SARS-CoV-2 virus RNA by one-step RT-PCR assay with Real-Time detection without isolation of RNA. Nasopharyngeal samples placed in **a Saline or PBS** transport media are introduced directly into the amplification reaction.
The Yzzy test Covid-19 Kit can 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

Clinical samples placed in a **Saline or PBS** transport media are used directly for subsequent amplification. Sodium azide (up to 0,05%) and proclines 300 and 950 (at final concentration up to 0,1%) are allowed as preservatives for transport media.

The YZZY test Covid-19 Express 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 Nucleocapsid (**N)** and RdRp genes (HEX/Yellow channel) and RNaseP internal control DNA (FAM/Green channel).
The system contains sufficient reagents for 100 RT-PCR reactions. The sensitivity of the system is 10 copies per reaction of SARS-COV-2' target. The Yzzy test Covid-19 Kit cross-reactivity with other respiratory viruses was tested by using in-silico BLAST analysis.

The sensitivity of express test system is 92% and specificity – 99% in comparison to non-express format.

Description

The YZZY test Covid-19 Express Kit detects two SARS-CoV-2 specific target sequences from the Nucleocapsid gene (N) and RdRp gene in one HEX/Yellow channel and one host specific target sequence from Homo sapiens RNase P gene (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 Control (NC), and Positive Control (PC-R) that monitors integrity of reagents and testing procedure accuracy.

REAGENTS AND MATERIALS

Materials Provided

The content of the YZZY test Covid-19 Express Kit indicated in Chart 1.

Chart 1. The content of the Yzzy test Covid-19 Kit.

Component	Quantity / Volume
1. RT-PCR Ex reagent	1 tube– 0.5 ml
2. Primers COVID-19 Ex	1 tube– 1.0 ml

3. Positive Control (PC-R)	1 tube– 0.2 ml
4. Negative control (NC)	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 Express Kit -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 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 Procedure

Sample Preparation and RNA Extraction

Naso-pharengial samples in Saline or PBS medium do not require isolation and are added directly into the amplification mixture

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** - is the total number of amplification reactions considering control samples).
3. Use a tube 1.5 – 2.0 ml for the “Master Mix” preparation:

Add 5x(N+1) µl of RT-PCR Ex reagent + 15x(N+1) µl of primers to a Master Mix tube.

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 5 µl of the PC-R to the marked PCR tube.

Negative control (NC): Add 5 µl of the NC to the marked PCR tube.

6. Add 5 µl of the Samples to the prepared PCR tube.

Chart 2. RT-PCR reaction summary.

Component	Volume
Master Mix	**20**
Sample/Control	**5**
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	2 min	1
Denaturation	95	5 sec	45
Annealing / Detection (FAM/HEX)	60	15 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 4.

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
NC	RT-PCR	–	Not analysed
PC	RT-PCR	+	+

4. 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	The value of "Ct" By FAM/Green reporter
Positive	+	+/–
Negative	–	+
Not valid	–	–

"-" indicates the absence of the "Ct" value, the amplification graph does not cross the threshold line
"+" Indicates the presence of "Ct", the amplification graph crosses the threshold line.
In the case of a non-valid sample, a PCR study of the corresponding sample is required to be re-done, starting with the RNA extraction step.

Important!

Visually inspect one-by-one the amplification curve for each sample that exhibits SARS-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 Guide

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
		Check the composition of transport media
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 reporter in NC	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.