INSTRUCTION FOR USE

(HANDBOOK)

CORONAVIRUS 2 (SARS-CoV-2)

REAL-TIME RT-PCR ONE-STEP QUALITATIVE KIT

Packing:01 boxes in total, boxes (50 tests/box) and OJ control box containing Positive and Negative Controls.

Expiration date: 12 months.

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INTENDED USE

The *Redcliffe Biosciences* SARS-CoV-2 RT-rPCR kit is an in vitro nucleic acid amplification test for SARS-CoV-2 virus detection in nasopharyngeal swab and oropharyngeal swab (l) based on RT-rPCR technique.

INTRODUCTION

Coronaviruses are a large family of viruses that are known to cause illness ranging from the common cold to more severe diseases such as Severe Acute Respiratory Syndrome (SARS-CoV) in 2002 and Middle East Respiratory Syndrome (MERS) in 2012. However, a novel coronavirus (nCoV) was identified in December 2019 in Wuhan, China. This virus can spread and cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. This is a new coronavirus that has not been previously identified in human<2>.

The World Health Organization (WHO) named the novel coronavirus (nCoV) was SARS-CoV-2. The genetic sequence of SARS-CoV-2 shows 80% similarly to SARS -CoV. Severe acute respiratory syndrome (SARS) is a recently emerged infectious disease with significant morbidity and mortality. An epidemic in 2003 of SARS affected 8,098 patients in 29 countries with 774 deaths. Vietnam had 63 infected cases and *5* deaths<6).

The newest research published on Lancet journal showed that clinical features of patients infected with SARS-CoV-2 including fever in 98%, cough in 76%, dyspnea in 55%, myalgia or fatigue in 44%, headache in 8%<3>.

Human-to-human transmission was published in two researches from patients in their families infected with SARS-CoV-2 on Lancet journal (*5* members in family infected with SARS-CoV-2 in Hong Kong) and New England Journal of Medicine journal (2 members from Chinese infected with SARS-CoV-2 moved to Vietnam)<3 4>.

There is currently no vaccine to prevent and specific antiviral treatment recommended for SARS-CoV-2 infection<5>.

PRINCIPLES OF PROCEDURE

The *Redcliffe Biosciences* SARS-CoV-2 R.T-rPCR kit uses real-time RT-PCR technique and TaqMan probe for SARS-CoV-2. There are three major processes: (1) specimen preparation to isolate SARS-CoV-2 RNA, (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of probes specific to the SARS-CoV-2 and SARS-like coronavirus.

Internal control (IC) allows monitoring from extraction procedure to result detection.

COMPONENTS AND REAGENTS

1. Amplification reagents *(store at -200C)*

- NA SARS-CoV-2 RT-rPCR Mix : 100 tests

2. Control sample *(store at -200C)*

- Positive control : 5x100µl
- Negative control : 1.5ml

SUGGESTED KITS

1. RNA extraction kit

- **Redcliffe Biosciences Viral RNA Extraction Kit - 50 tests/kit**
- QIAGEN'S viral RNA extraction kit.

MATERIALS AND EQUIPMENT REQUIRED

- Real-time PCR machine and computer system.
- Microcentrifuge, clean cabinet, micropipettes 10µl.
- Consumables: Disposable powderless latex gloves, aerosol barrier pipette tips.

WARNINGS AND PRECAUTIONS

- Read the instructions carefully before processing samples.
- For in vitro diagnostic use.
- Users need to be trained in molecular biotechnology.
- Reagents need to be preserved according to official instruction. Avoid contaminating microorganism with reagents.
- Do not mix reagents from two or more kits together. Do not use out-of-date kits.
- PCR working area needs to be differentiated from electrophoresis working area.
- Do not use common equipment in distinguished function regions. Ensure that DNases are absent in all of machines and equipment.
- Working surfaces and micropipettes should be kept clean and wiped periodically.
- UV treatment in PCR workstation for 15 minutes before and after working.
- Wear disposable gloves when handling samples and kit reagents. Change gloves after each process.
- Change new tips for each sample.
- Positive control and negative control must be ready to use.

PROTOCOL

1. Specimen collection and preparation:

- Take a sample of nasopharyngeal swab and oropharyngeal swab then put into a tube containing the specimen preservation solutions.

Note:

- The specimen is stored, packed and transported to the laboratory according to WHO

- Class II biological safety cabinet (BSC-II) is used for handling activated specimens.
- Healthcare workers must wear personal protective equipment (medical gloves and masks, goggles, gowns or aprons protect the skin and/or clothing, safety shoes, long-sleeved coats). When the sample is collected with aerosol procedure, personnel must wear N95 respirators, which has NOSH certified, NlOSH certified FFP2 or equivalent.

2. **RNA extraction:**

- See the "User's Manual" of the kit Redcliffe's Viral RNA Extraction VA.A92-002B, VA.A92-002G.
- Other kits made by Qiagen, Roche and so on are possible to use.

3. **Real-time RT-PCR:**

- Real-time RT-PCR is very sensitive to contamination from outside PCR products.
- It's very important to set up your reactions on ice.
- Only use an adequate amount of PCR mix. The remains of PCR mix should be stored in preservative condition before continuing the real-time RT-PCR process.
- Before and after doing real-time RT-PCR, centrifuge the tube to gather the liquid in the bottom of the tube.
- In case of using the real-time PCR machine with bottom-tube reader system: Label the sample's code on the lid or on the body near the lid. In case of using the real-time PCR machine with lid-tube reader system: Label the sample's code on the body near the lid.
- Take exactly 5µl positive control, negative control or RNA sample in each SARS- CoV-2 RT-rPCR Mix. Be sure that all the solution in tips is completely transferred to the tubes. Avoid external contamination risks: Open only the lid of the tube containing testing sample then tightly close the lid of tube immediately. Put the tubes in real-time PCR machine after centrifugation.

Turn on the real-time PCR machine. Tum on the computer and make real-time PCR program ready to run. Check the connection between real-time PCR machine and computer carefully (see the User's Manual of real-time PCR machine). When the "Heat lid" reaches to 105°C, the thermal cycle start working.

- Set the sample positions by "Plate setup" on PCR program that identical to the real sample positions on the real-time PCR machine.
- For samples: Select "Unknown" type. Set the name or number corresponding to the sample's code.
- For positive control and negative control: It is preferred to select the "Unknown" type. Do not select "PTC -Positive Control" and "NTC -Negative Control". Set the names "Positive Control", "Negative Control" for appropriate controls.
- Choose "FAM", "TEXAS RED" and "HEX" channels for samples, positive control, and negative control respectively.
- "FAM" channel: detect SARS-CoV-2 target sequence. "TEXAS RED" channel: detect SARS-like coronaviruses target sequence. "HEX" channel: detect internal control (IC) sequence.
- Set up "Protocol" program to run the real-time PCR machine: 1cycle for 10 minutes at 50℃, 1cycle for 5 minutes at 95°C; 40 cycle for 15 seconds at 95°C, 45 seconds at 55°C (read results at this step).
- Save data to the computer. Do not save data to the administration hard disk.
- Run real-time RT-PCR program. See the User's Manual of the machine to select the best program.
- Testing for agents have same PCR cycling protocol can be performed at the same time. If detection channels of agents are different, choose the appropriate channel for each

agent.

QUALITY CONTROL

- One negative control, one positive control, and one internal control are processed with each run.

INTERNAL CONTROL

- The kit use the RNAse P (RP) gene as an internal control in order to control the quality of the sample and real-time RT-PCR assay performance.

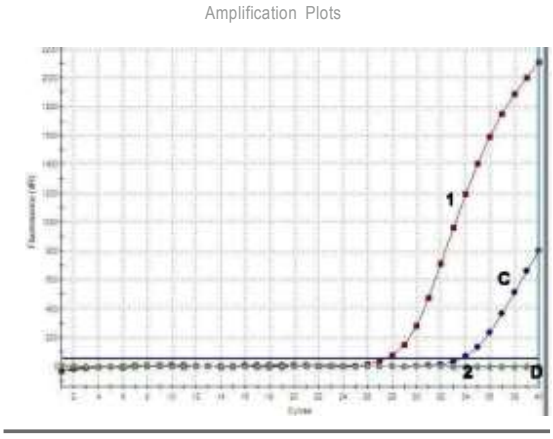
INTERPRETATION OF RESULTS

- Positive, negative and internal control analysis: Choose the well containing positive control and negative control, select FAM, TEXAS RED channels. The tests are considered to be accepted and continued to the next steps if FAM and TEXAS RED fluorescent signal lines of positive control are linear curve over baseline (called positive line), FAM and TEXAS RED fluorescent signal lines of negative control are negative. Other cases: See "Troubleshooting" or contact your supplier.
- Sample analysis: Choose FAM chanel for SARS-CoV-2, TEXAS RED chanel for SARS-like coronaviruses and HEX chanel for internal control. The curves can be analyzed at dR (baseline-corrected raw fluorescence) or R (raw fluorescence) mode (See the Instruction Manual of the machine). Select each sample and analyze data at raw fluorescence mode for best results. Ifthe sample has clear positive curve starts before/at the 36th cycle correspond to which agent, conclude that "The sample is infected with SARS-CoV-2 virus". Ifthe sample has negative curve for all agents and positive HEX fluorescent signal line starts after the 35th cycle, conclude that "Viruses are not found in the sample". Other cases: See "Troubleshooting" or contact your supplier. To conclude the negative samples, the HEX fluorescent signal of internal control must be positive.

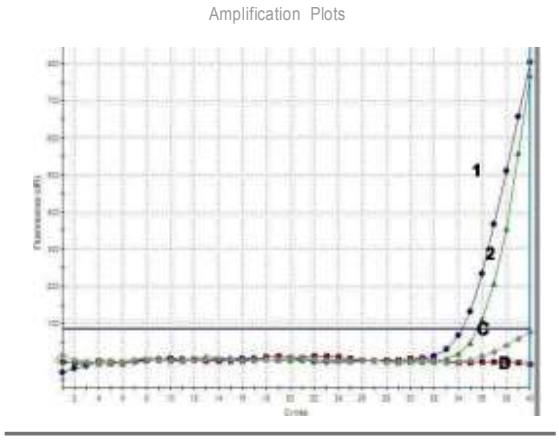
Conclusion	SARS-CoV-2 (FAM)	SARS-like coronaviruses (TEXAS RED)	IC-RP (HEX)
SARS-CoV-2	+	+	+
SARS-like coronaviruses	-	+	+
Negative	-	-	+
Invalid sample	-	-	-

- Result printing: Choose the graphs of the negative control, internal control and samples. Copy and paste the graphs to the resulting page, label the "Internal control", "Negative control" and "Sample" in each curve.
- Real-time PCR experiment is depended on more factors. To get the more precise analysis, we have to divide the lines into more groups. Sometimes we have to analyse each sample. Every experiment can have more baseline positions however it must ensure the required standards. This is a general analysis method. To achieve the correct results, follow the User's Manual of the machine is highly recommended.
- Positive samples and positive RNA could be stored at -20°C or -70°C to use as the positive control in the next test.

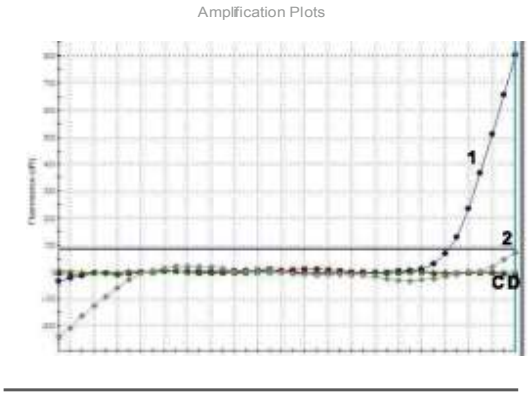
REFERENCE RESULTS



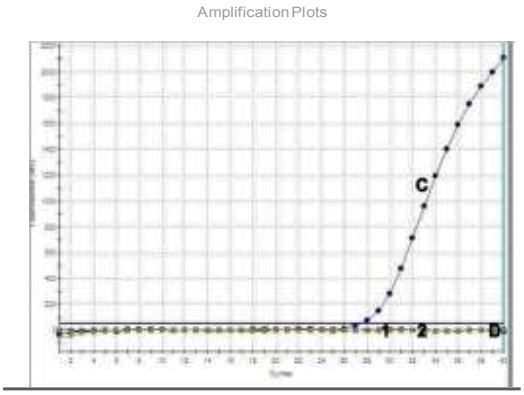
SARS-Iike coronaviruses is strong positive, SARS-CoV-2 is negative.
Conclusion: "The sample is infected with SARS-like coronavlruses"
l:TEXAS RED, 2:FAM, C: Internal control, **D:** Negative control



SARS-CoV-2 is positive, SARS-like coronaviruses is positive.
Conclusion: "The sample is infected with SARS-CoV-2"
l:FAM, 2: TEXAS RED, C: Internal control, **D:** Negative control



SARS-CoV-2 i s negative, SARS-like coronaviruses is negative, internal control is positive after 35th cycle.
Unable to conclude
1:Positive control, 2: Internal control
C: SARS-CoV-2, D: SARS-like coronaviruses



SARS-CoV-2 and SARS-llke coronaviruses are negative, internal control is positve.
Conclusion: "The sample is negative"
l:SARS-CoV-2, 2: SARS-like coronaviruses,
C: Internal control, **D:** Negative control

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KEY PERFORMANCE CHARACTERISTICS

LIMIT OF DETECTION

SARS-CoV-2 positive sample from were used to determine the limit of detection. The positive sample with a concentration of 10^4 copies/reaction was diluted to 10^1 copies/reaction. The criteria used for evaluation are Ct (Threshold cycle - the period in which a positive signal crosses the background signal).

After serial dilutions from 10^4 copies/reaction to 1 copy/reaction (30 replicates/concentration). At concentrations from 10^4 copies/reaction to *5* copies/reaction, these concentrations still were positive. At a concentration of 1 copy/reaction, it was positive at 11replicates (36.67%).

Concentration (copies/reaction)	Replicate	Positive	Ratio (o/o)
10^4	30	30	100
10^3	30	30	100
10^2	30	30	100
10^1	30	30	100
5	30	30	100
1	30	19	63.33

Therefore, the limit of detection was *5* copies/reaction

SPECIFICITY

Evaluation of the specificity was done with the common pathogens of viruses and bacteria in human including SARS-CoV, influenza A/Hlpdm09, influenza A/H3, influenza A/HS and influenza B. In particular, all strains of viruses and bacteria have been identified positive with their specific kits.

Pathogens	SARS-CoV-2 *(FAM)*	SARS-like coronaviruses *(TEXAS RED)*	IC- RP *(HEX)*
SARS-CoV-2	+	+	+
SARS-like coronaviruses	-	+	+
SARS-CoV	-	-	+
influenza A/Hlpdm09	-	-	+
influenza A/H3	-	-	+
influenza A/HS	-	-	+
Influenza B	-	-	+

ACCURACY

The reaction accuracy was evaluated through intra-assay and inter-asay. We conducted on IDT SARS-CoV-2 amplicon with concentrations 107 10^6, 10^5, 104, 10^3 copies/ml, IDT positive control (code: 1000662S) with concentrations 10^5, 104, 10^3 copies/ml, sample positive from National Institute of Hygiene and Epidemiology and negative sample. For intra-assay, each concentration was repeated S times. For inter-asay each concentration was repeated S times at S different times. Coefficient of variation (CV) is calculated based on Ct value.

The results of intra-assay of the *Redcliffe Biosciences* SARS-CoV-2 RT-rPCR Kit on serial dilution in S times showed at the following results:

The results of intra-assay of the *Redcliffe Biosciences* SARS-CoV-2 RT-rPCR Kit on serial dilution in S times showed at the following results:

Serial Dilution (copies/ml)	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 4**	**Replicate 5**	**CV intra-reaction (%)**
Amplicon 10^3	29.65	29.75	30.24	29.74	30.76	1.57
Amplicon 10^4	26.56	26.87	26.57	27.68	26.97	1.70
Amplicon 10^5	22.76	23.78	22.87	22.54	22.53	2.25
Amplicon 10^6	20.57	19.97	21.44	20.76	19.35	3.89
Amplicon 10^7	16.86	16.58	17.69	17.46	16.76	2.80
Positive sample from National institute of hygiene and epidemiology	26.86	26.63	27.46	27.75	26.86	1.74
IDT Positive control 10^5	22.46	23.75	22.78	22.66	22.69	2.22
IDT Positive control 10^4	26.86	26.46	26.97	27.53	26.95	1.42
IDT Positive control 10^3	29.54	29.86	30.36	29.75	30.54	1.341
Negative control	Negative	Negative	Negative	Negative	Negative	

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The results of inter-assay of the *Light Power SARS*-Co V-2 1sT-rPCR Kit on serial dilution in 5 times at 5 different times showed at the following results:

Samples (copies/ml)	Day 1	Day 2	Day 3	Day 4	Day 5	CV inter-assay %)
Amplicon 10^3	30.64	29.54	30.86	29.33	30.64	2.35
Amplicon 10^4	26.56	26.79	27.44	27.67	26.76	1.78
Amplicon 10^5	22.96	23.46	22.86	22.34	22.64	1.82
Amplicon 10^6	19.79	20.97	19.35	20.67	19.87	3.32
Amplicon 10^7	16.08	16.57	17.89	17.65	16.68	4.51
Positive sample from National institute of hygiene and epidemiology	26.36	26.53	26.86	27.31	26.75	1.35
IDT Positive control IDT 10^5	22.87	23.44	22.35	22.97	22.22	2. 17
IDT Positive control IDT 104	26.57	26.97	27.35	27.65	26.64	1.71
IDT Positive control IDT 10^3	30.06	29.45	30.97	29.33	30.53	2.32
Negative control	Negative	Negative	Negative	Negative	Negative	

TROUBLESHOOTING

Problem 1: All samples are positive including the negative control.

- **Reason 1:** External RNA or other PCR products in the working area presents in this experiment or cross-contamination among the samples. **Solution:** Use UV light and clean the working area with Javel solution. Do the experiment again.
- **Reason 2:** External RNA or other PCR products are present in the kit. **Solution:** Use a new kit and follow the instruction in reason 1.

Problem 2: All of the samples are negative including the positive control.

- **Reason 1:** Extraction kit and/or real-time RT-PCR kit have got the errors. **Solution:** Use a new kit.
- **Reason** 2: Real-time PCR machine has got the errors. **Solution:** Repair Real-time PCR machine or use another Real-time PCR machine.
- Inthese cases, the result must be totally eliminated and the experiment needs to be performed again after solving the problems.

Problem 3: All of the samples are negative even internal control but the positive control is positive

- **Reason:** Extraction kit is failed. **Solution:** Change a new extraction kit.
- In this case, the result must be totally eliminated and the experiment needs to be performed again after solving the problems.

Problem 4: Positive control is negative, the internal control in sample is positive.

- **Reason:** Positive control got an error. **Solution:** Change a new positive control.
- Inthis case, the result could be used. The above solution should be used in the next experiment.

Problem 5: Negative control is positive.

- **Reason:** External RNA or other PCR products in working area are present in negative control or there is cross-contamination between negative control and

positive sample. **Solution:** Use UV light and clean working area with Javel solution.

- Do the experiment again with new negative control.

Problem 6: All of the curves in the final graph are abnormal.

- **Reason 1:** The type of PCR mix tube is not appropriate for the PCR machine. **Solution:** Use another type of tube.
- **Reason 2:** The real-time PCR machine is broken. **Solution:** Repair the machine or use another machine

Note: The problems could be caused by many reasons. Please contact the supplier to be supported.

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