AddMedi SARS-CoV-2 RT-qPCR Kit

Instruction for Use (EN)

REF ADM-SC100 CE IVD

Real-time PCR Test for Professional Use

This instruction must be read carefully prior to use. Reliability of assay results cannot be guaranteed if there is any deviation from the instructions.

1. INTENDED USE

The AddMedi SARS-CoV-2 RT-qPCR Kit (CE-IVD) is intended to be used to attain qualitative detection of new Corona virus (SARS-CoV-2). Viral nucleic acid extracted from Bronchoalveolar lavage fluid, sputum, throat & nasal swabs, virus preservation buffer, universal transport media (UTM), Serum and others from patients in association with a CE-IVD extraction system and the designated RT-qPCR platforms. The kit is intended for use by professional personnel of clinical laboratory under decent laboratory practice.

2. COMPONENTS OF KIT CONT

The Product "AddMedi SARS-CoV-2 RT-qPCR Kit" is packaging for 100 tests/kit. The insertion of the components is below-

- ☞ 5X RT-qPCR buffer (Cap label: 5X Buffer): 1 vial is containing 400㎕
- ☞ 20X Enzyme solution (Cap label: 20X ES): 1 vial is containing 100㎕
- ☞ 4X Oligo mixture (Cap label: 4X OM): 1 vial is containing 500㎕
- ☞ Positive control (Cap label: PC): 1 vial is containing 100㎕ CONTROL +
- ☞ Negative control (Cap label: NC): 1 vial is containing 100㎕ CONTROL -

3. PRINCIPLE OF TESTING PROCEDURE

The principle of test is based on TaqMan Real-Time qPCR. After open the Kit box, resuspend the 5X RT-qPCR buffer (5X Buffer, 400㎕), 20X Enzyme Solution (20X ES, 100㎕), 4X Oligo mixture (4X OM, 500㎕), Positive control (PC, 100㎕), and DEPC water for Negative Control (NC, 100㎕). Avoid generating air bubbles. Then Aliquot the 5X RT-qPCR buffer (4㎕), 20X Enzyme Solution (1㎕), 4X Oligo mixture (5㎕), RNA template (5 or 10㎕), PC (10㎕) and remain volume should be adjusted with DEPC to the reaction volume of 20㎕ into the PCR tube or plate for the chosen qPCR platform. Aliquot into wells according to the number of samples to be tested, include one well for the positive control (PC) and one well for the DEPC water for negative control (NC). All preparation reaction mixture transfer to the Sample Processing Area. Add 5㎕ or 10㎕ (here no need adjusted with DEPC water) RNA temple of the following into the appropriate wells according to plate setup with the Sample(s), Positive Control and Negative Control. After adding the samples, positive control, and negative control cover the lid immediately. Spin down briefly using a centrifuge to remove air bubbles. Transfer the mixture to amplification area. Place the tubes on the sample holder in the instrument. Setup the test panel according to the positions of the RNA samples, positive control, and negative control. Select the detection channels as following: Select FAM (S gene), HEX (RdRp gene), and all channels for IPC to detect SARS-CoV-2 virus RNA. The Product "AddMedi SARS-CoV-2 RT-qPCR Kit" is based on non-ROX option.

4. REAGENT STORAGE, SHELF LIFE AND HANDLING

- All reagents should be stored at -10°C ~ -30°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Do not repeated thawing and freezing more than 5 times and light should be avoided, as this may reduce the sensitivity of the assay.
- All reagents should be handled on ice during preparation of mixture.
- Oligo Mix (OM) should be stored in the dark.

5. ADDITIONALLY REQUIRED MATERIALS AND DEVICES

- Biological cabinet
- RNA extraction kit
- Cryo - container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and freezer
- Vortex mixer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipettes (0.5㎕ - 1000㎕)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "Eppendorf" type tubes

6. WARNINGS AND PRECAUTION

Carefully read this instruction before starting the procedure.

- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas:
  i) Isolation of the RNA/ DNA and
  ii) Amplification / detection of amplification products.
- Pipettes, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols

7. LIMITATIONS EXP

- It must be kept at the storage temperature until expiry date. (Storage temperature -10°C ~ -30°C, expiry date 18 month after manufacturing, 20 days after opening)
- It should be kept away from light.
- Use on ice during the test.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay is not to be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the SARS-CoV-2 virus should be interpreted in consideration of all clinical and laboratory findings.

8. SAMPLE COLLECTION, STORAGE AND TRANSPORT

- Collected samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. PROCEDURE

9.1. Viral nucleic acid extraction

Different brand viral nucleic acid extraction kits are available. You may use your own extraction systems or the commercial kit based on the yield. For the viral nucleic acid extraction, please comply with the manufacturer's instructions. The recommended Extraction kit is as follows:

Viral nucleic acid extraction kit	Cat. No.	Manufacturer	Remark
AddPrep Viral Nucleic Acid Extraction Kit	10034	AddBio Co. (www.addbioinc.com)	CE-MARKED

9.2. Reaction mixture and PCR conditions

Reaction mixture		PCR condition		
Components	Volume	Temp.	Time	Repeat Cycles
5X RT-qPCR buffer (5X Buffer)	4 ㎕	50°C	20 min	1
20X Enzyme Solution (20X ES)	1 ㎕	95°C	10 min	1
4X Oligo Mixture (4X OM)	5 ㎕	95°C	25 sec	40
RNA Template (or PC, NC)	10 ㎕	60°C	50 sec	
Total reaction volume	20 ㎕			

9.3. Result interpretation

The results interpretation of investigate the amplification curve of the option with non-ROX channel. If Ct ≤ 38, it indicates that the detection is valid, and users can continue the subsequent analysis:

a) If a typical S-type amplification curve is detected by the FAM (S gene) channel, with Ct ≤ 38, it indicates that SARS-CoV-2 virus is positive.

b) If a typical S-type amplification curve is detected by the HEX (RdRp gene) channel, with Ct ≤ 38, it indicates that SARS-CoV-2 virus is positive.

If the Internal Positive Control (IPC) a typical S-type amplification curve is detected by the Cy5 *(Brassica rapa)* channel failed to detect Ct or Ct > 38, it indicates that there is an inhibitory reaction from the interfering substances. Users have to repeat the experiment. For every reaction of positive samples and negative sample, IPC will be amplified. If the IPC is not amplifying, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment. (If the retest result is still invalid, please contact the manufacturer.)

10. RESULT ANALYSIS

All the results are based on Ct values that automatically calculated by software.

10.1. Fluorophore and cut-off value

Target	Fluorophore.	Cut-off of Ct value
S gene	FAM	<38
RdRp gene	HEX	<38
IPC	Cy5	<38

* Refer to the appropriate threshold line for each instrument

10.2. Interpretation of sample results

Sample	S gene	RdRp gene	IPC	Result
	FAM	HEX	Cy5	
Negative	-	-	+	Valid, SARS-CoV-2 virus not detection
	-/+	-/+	-	Invalid, re-test
Positive	+	-	+	Invalid, re-test
	-	+	+	Invalid, re-test
	+	+	+	Valid, SARS-CoV-2 virus detection
	-/+	-/+	-	Invalid, re-test

* Cut off: < 38 Ct

10.3. Results trouble shooting

Problems	Probable cause	Recommendation
Cannot see any signal in all channel including positive control	Wrong operation of instrument	Please check Real-time PCR condition and run the assay under correct setting
	Incorrect preparation of mixture	Please check all components and repeat assay
	Not available storage condition	Repeat the assay using fresh reagents
False positive at the negative control	Carry-over contamination	Discard all the components of assay. Repeat the assay using new components
Not acceptable positive control	Degradation of positive control	Aliquot when thaw positive control. Avoid repeated freezing and thawing
	Incorrect preparation	Please confirm the protocol and repeat assay.
No appearance or high Ct value of IPC	High concentration of sample	Retest after diluting the DNA using nuclease free water

10.4. Test instruments

Equipment	Company	Cat. No.
BioRad Real-time PCR machine	BioRad Laboratories Inc USA	CFX96 Real-Time PCR System
ABI Real-time PCR machine	Thermo Scientific, USA	Thermo Scientific, USA

11. PERFORMANCE EVALUATION

11.1. Limit of Detection (Analytical Sensitivity)

LOD is determined as copy number of diluents that showed 100% detection up to 50 copies of SARS-CoV-2 virus. Whereas, S gene was detected 33.3% and RdRp gene was detected 45.8% as of 15 copies for lower detection assay.

11.2. Analytical Specificity with interfering substances

The AddMedi SASR-CoV-2 RT-qPCR Kit was affected by the interfering substance using a standard substance as the minimum detection limit concentration. One lot was used, and 3 repetitions were performed on one machine. As a result, it was confirmed that there was no effect on the test results.

11.3. Diagnostic Specificity (Cross reaction)

The cross-reaction test results are performing with pathogens (25 virus and bacteria) and virus-positive substances (1 sample). It was confirmed that positive samples are detected and where negative samples not detected. The internal positive control (IPC), which can confirm the inhibition of the PCR reaction, was detected in all reaction solutions that remarked effectiveness of the test could be confirmed. The test was confirmed 3 times repeated using one lot in an equipment.

No.	Microorganism
1	Twist Synthetic SARS-CoV-2 (EPI_ISL_418227) RNA
2	Twist Synthetic Influenza H1N1 (2009) RNA
3	Twist Synthetic Influenza H3N2 RNA
4	Twist Synthetic Influenza B RNA
5	Twist Synthetic Human coronavirus 229E RNA
6	Twist Synthetic Human coronavirus OC43 RNA
7	Twist Synthetic SARS coronavirus Tor2 RNA
8	Twist Synthetic MERS coronavirus 2c EMC/2012 RNA
9	Human metapneumovirus
10	Human Coronavirus NL63
11	Human Respirovirus 3 (Parainfluenza virus 3)
12	Human Respirovirus 1 (Parainfluenza virus 1)
13	Human Rhinovirus 14
14	*Legionella pneumophila subsp. Pneumophila*
15	*Legionella pneumophila subsp. Fraseri*
16	*Streptococcus pyogenes*
17	*Mycobacterium smegmatis*
18	*Mycobacterium diernhoferi*
19	*Mycobacterium terrae*
20	*Mycobacterium flavescens GTC 608*
21	*Shigella flexneri*
22	*Shigella boydii*
23	*Salmonella enterica*
24	*Salmonella bongori*
25	*Vibrio parahaemolyticus*
26	*Yersinia enterocolitica*

11.4. Test of Repeatability

Repeatability test with the AddMedi SASR-CoV-2 RT-qPCR Kit, the concentration of each sample is 3 repeats (3X LoD, 1X LoD, 0.5X LoD), and two expert researchers used one lot with same experiment in twice per experiment (am/pm) for 20 days. As a result of the experiment, 100% of all samples were detected in moderate positive (3X LoD) and 100% in low positive (1X LoD). At the below concentrations the minimum detection limit (0.5X LoD), 37.5% of the S gene and 42.5% of the RdRp genes were successfully detected. As a result, the repeatability of the AddMedi SASR-CoV-2 RT-qPCR Kit was confirmed within 5% of CV.

11.5. Test of Reproducibility

As a result of the reproducibility test experiment, 100% of all samples were detected in sensible positive (3X LoD), and 100% was detected in near to the ground positive (1X LoD). Consequence of the results, 0.5X LoD was detected 37.8% and 31.1% of S gene and RdRp gene, respectively. The reproducibility of the AddMedi SASR-CoV-2 RT-qPCR Kit was confirmed within 5% of CV.

11.6. Clinical Sensitivity and Specificity

Nasopharyngeal Swabs (Target: S gene and RdRp gene)		Test Results	
		Positive	Negative
AddMedi SASR-CoV-2 RT-qPCR Kit	Positive	48	0
	Negative	2	30
SARS-CoV-2 Detection Sensitivity = 96.00% (48/50) (95% CI: 86.54% ~ 98.90%)			
SARS-CoV-2 Detection Specificity = 100.00% (30/30) (95% CI: 88.65% ~ 100.00%)			

12. Bibliography

- World Health Organization (2016) Guidelines on care components of infection prevention programs at the national and acute healthcare facility level.
- "Interim laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)"
- Clinical management of severe acute respiratory infection when novel coronavirus(nCoV) infection is suspected. Interim guidance. WHO. 2020.
- Policy for Coronavirus Disease-2019 Testes During the Public Health Emergency. USFDA.

13. References

1. Seyed A.H., Saghar S., Hamed G.M., Majid G. and Amir A. 2021. High prevalence of SARS-CoV-2 and influenza A virus (H1N1) coinfection in dead patients in Northeastern Iran. Journal of Medical Virology. 93:1008-1012.
2. Seyed A.H., Amirhosein K., Hamed G.M., Majid G., Mohammadreza T., Hasan N.A. and Amir A. 2020. Development of a PCR-RFLP method for detection of D614G mutation in SARS-CoV-2. Infection, Genetics and Evolution. 86:104625.
3. Ayad M. A., Mohammed A., Mohammed H.F., Hassan M.T. and Hassan M.R. 2020. SARS-CoV-2 Reinfection in Patients Negative for Immunoglobulin G Following Recovery from COVID-19. MedRxiv and BioRxiv. doi: https://doi.org/10.1101/2020.11.20.20234385.

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▪ Description of Symbol Used

Symbol	Symbol Title	Symbol	Symbol Title
	Manufacture	REF	Catalog number
CE	CE Mark	⚠	Caution
EXP	Use-by / Expiration Date	IVD	In Vitro Diagnostic Medical Device
	Consult Instructions for Use		Potential Biohazard
LOT	Batch Code	CONTROL -	Negative Control
	Temperature Limit	CONTROL +	Positive Control
CONT	Contains / Contents	EC REP	Authorized representative in the European Community

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