RT-RAA Nucleic Acid Amplification Kit（Fluorescent Method）Instruction Manual

[Product] Universal Name: RT-RAA Nucleic Acid Amplification Kit (Fluorescent Method).

[Specifications] 48 Tests/Kit、96 Tests/Kit

[Intended Use] This kit is for scientific research purpose；it can be used to amplify different nucleic acid. The nucleic acid amplification result can be verified by fluorescent probe.

[Main Components]

Component	Volume	48 Tests/Kit	96 Tests/Kit
Purified Water	1500 μL/Tube	1 Tube	1 Tube
Buffer VI	1500 μL/Tube	1 Tube	2 Tubes
Magnesium Acetate Ⅰ	600 μL/Tube	1 Tube	1 Tube
RT Reaction Unit(F)	12T/Pack	4 Packs	8 Packs
Forward Primer of Positive Control(F)	25 μL/Tube	1 Tube	1 Tube
Reverse Primer of Positive Control(F)	25 μL/Tube	1 Tube	1 Tube
Probe of Positive Control(F)	10 μL/Tube	1 Tube	1 Tube
Positive Control(F)	25 μL/Tube	1 Tube	1 Tube
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Note: Self-supplied Materials: Nucleic Acid Extraction Kit,96-well plate.

[Detection Principle]

This kit adopts RAA (Recombinase Aided Amplification) to amplify virus RNA, the amplification result can be verified by real-time fluorescent probe.

This kit performs the reverse transcription process and the amplification process simultaneously. In constant temperature (normally 39 ℃), within 10-30 min，the sample RNA as template can be amplified.

This Kit can be used in scientific research. The length of primer needs to be 30-35 bp, the length of target nucleic acid to be amplified needs to be 80-500 bp. The length of probe needs to be 46-52 bp, fluorescence group and quench group are labeled on both sides of the middle THF site, and at least 30 bp is required for the 5' end to reach the THF site. At least 15 bps is needed for the THF site to reach the 3' end, and the 3' end is modified with the blocking group to ensure the rapid, sensitive and effective amplification reaction

[Storage Conditions and Expiration Date]

12 months when stored at -20±5℃ in the dark and avoid repeated freeze-thaw.Reaction unit can be stored for 1 month after unsealing.

1 month when stored at 2~8℃ in the dark..

[Sample Requirements]

Sample collection: Collect sample through normal collection method.

Sample Transportation: Using Ice box or bubble chamber with ice to transport samples.

[Detection Instruments]

Isothermal Nucleic Acid Amplification Detector RAA-F1620(or RAA-F1610),Sample Pretreatment System RAA-B6108 manufactured by Wuxi Qitian Biological Science Instrument Co., Ltd.

Mini centrifuge PCR detector for detection of FAM fluorescence.

[Test Method]

1.Nucleic Acid Extraction---【Nucleic Acid Extraction Area】

Nucleic acid extraction kit or automated nucleic acid extraction instrument can be used for nucleic acid extraction of samples to be tested. For specific extraction methods, please follow the corresponding product instructions.

2.The steps of Isothermal Nucleic Acid Amplification Detector RAA-F1620(or RAA-F1610)

2.1 Reagent Preparation---**【Reagent Preparation Area】**

(1) Sample Pretreatment System RAA-B6108 and Isothermal Nucleic Acid Amplification Detector RAA-F1620 (or RAA-F1610) are started 30 minutes before the detection.

(2) The aluminum foil packing reagents are taken out from the kit, tear the foil, take out tubes needed in the detection, place them on 96-well plate, and take out Buffer VI. The remaining reaction tubes should be immediately sealed in an aluminum foil self-sealing bag and stored at -20±5 ℃.

(3) Reaction system preparation：prepare the reaction system according to the following table

The reaction system for each test is as follows:

Component	Volume（μL）
Buffer VI	25
Forward Primer（10μM）	2.1
Reverse Primer（10μM）	2.1
Probe（10μM）	0.6
Purified Water	15.2-n
Total	45-n

Note：①n means the addition volume of RNA template，if the template concentration is high, the recommended addition volume of template is 1μL.
②Purified water can be used as negative control.
③If the number of RNA template to be tested is X, it is recommended to prepare X+2 tubes of mix to subpackage（including negative control and positive control).

(4) The above mixture was mixed by hand and centrifuged briefly, add (45-n) μL mixture to reaction tubes, the lyophilized powder is fully and evenly dissolved by gently flicking with hand(**Note：** this step cannot finished with Votex Oscillator),

collect the liquid to the bottom of the tube with brief centrifugation.

2.2 Sample Addition---**【Sample Preparation Area】**

Open the reaction tube lid，add 5μL magnesium acetate Ⅰ in the tube lid，then add n μL negative control or RNA of sample to be tested to reaction tubes respectively，keep in mind that the pipette tip should be placed below the liquid level to add the sample. Add one sample, close the lid, and then add the next sample, the sequence of addition is: negative sample/negative control, RNA of sample to be tested, positive sample/positive control, mix well and centrifuge to collect.

2.3 Amplification Detection---**【Amplification Detection Area】**

Symmetrically place the reaction tubes into the RAA-B6108，long-press the “pretreatment” key (7 min countdown will appear on the window) for pre-amplification, take out the tubes after the end of run with buzzer.

Place the tubes into RAA-F1620 (or RAA-F1610), wherein the reaction temperature is set at 39 ℃ for 30 min and observe the result in real time.

After the end of the reaction, take out the reaction tube (closed tube) and put them into the plastic bag according to the contamination source treatment.

3. The steps of PCR detector for detection of FAM fluorescence

3.1 The experiment preparation

Open the water bath and set the temperature at 39℃ before the experiment.

3.2 Reagent Preparation---**【Reagent Preparation Area】**

(1) The aluminum foil packing reagents are taken out from the kit, tear the foil, take out PCR tubes needed in the PCR detector, place them on 96-well plate, and take out Buffer VI. The remaining reaction tubes should be immediately sealed in an aluminum foil self-sealing bag and stored at -20±5 ℃.

(2) Reaction system preparation：prepare the reaction system according to the following table

The reaction system for each test is as follows:

Component	Volume（μL）
Buffer VI	25
Forward Primer（10μM）	2.1
Reverse Primer（10μM）	2.1
Probe（10μM）	0.6
Purified Water	15.2-n
Total	45-n

Note：①n means the addition volume of RNA template，if the template concentration is high, the recommended addition volume of template is 1μL.
②Purified water can be used as negative control.
③If the number of RNA template to be tested is X, it is recommended to prepare X+2 tubes of mix to subpackage（including negative control and positive control).

(3) The above mixture was mixed by hand and centrifuged briefly, add (45-n) μL mixture to reaction tubes, the lyophilized powder is fully and evenly dissolved by gently flicking with hand(Note：this step cannot finished with Votex Oscillator), collect the liquid to the bottom of the tube with brief centrifugation.

(4) Transfer the solution in step (3) into the corresponding PCR tube matched to PCR detector successively.

3.2 Sample Addition---**【Sample Preparation Area】**

(1) Open the cover of PCR tube, add 5μL magnesium acetate I to the tube cover, and then add N μL quality control substance or RNA of sample to be tested to each reaction tube. Add samples in the sequence of negative quality control substance, RNA of sample to be tested and positive quality control substance.

(2) Take the PCR tube into hand-held centrifuge for short centrifugation, and then take the magnesium acetate I into the tube to be centrifuged

(3) Hit the wall of PCR tube with a little force by fingers,fully mix the reagent with the sample and place the reagent in a hand-held centrifuge for a short centrifuge (note: if there are too many bubbles in the reaction tube, centrifuge shall be used to remove them, so as to avoid affecting the detection results of the PCR instrument).

(4) Take the PCR tube to be placed in a constant temperature water bath at 39℃ for 7 minutes.

(5) Repeat step (3) after the water bath time.

3.3 Amplification Detection---**【Amplification Detection Area】**

(1) Put the PCR tube into the PCR detector, set the reaction program according to the following table and run it.

Steps	parameters	Cycle number
Pre degeneration	39℃ 30s	1
Extension	39℃ 30s (The Fluorescent Collecttion)	50

(2) After the reaction, take out the reaction tube (closed tube) and put it into plastic bag, and treat it according to the source of pollution.

4. Result analysis

4.1 Analysis of the result of Isothermal Nucleic Acid Amplification Detector RAA-F1620(or RAA-F1610)

(1) After the reaction is over, set the slope to 20, and the instrument will automatically analyze the data and present the result on screen (if the instrument is RAA-F1620).

(2) Set the threshold to 300, after the reaction is over, the instrument will automatically analyze the data and present the result on screen (if the instrument is RAA-F1610).

(3) The instrument has Channel 1 (FAM channel), which is the detection channel. When the Channel 1 shows positive, the sample in question represents a positive result, when the Channel 1 shows negative, the sample in question represents a negative result.

4.2 Results analysis of PCR detector that can detect FAM fluorescence

According to the results of PCR detector, the negative was not amplified, and the positive was amplified significantly.

5. Quality Control

The positive control is positive; Negative quality control is negative.

If the test results do not meet the requirements of (1), the experiment shall be deemed to be invalid, and the deviation of instruments and reagents shall be checked, and the test shall be conducted again after confirmation.

[Limitations of the Kit]

1.This kit is only used in the laboratory and is suitable for amplification of various RNA.

2.Inappropriate sample collection, storage and processing may lead to wrong results in the detection after amplification.

3.Unverified interferences or RAA inhibitors may lead to false negative results.

4.Laboratory environmental contamination, reagent contamination or cross-contamination of samples will lead to false positive results after amplification.

5.Improper transportation and storage of reagents, inaccurate preparation of reagents or inadequate mixing of reagents will lead to the decline of reagent detection efficiency, false negative or inaccurate detection results.

[Product Performance Index]

The plasmid with 10 copies / test can be stably amplified at 39 ℃; the LOD of the sample RNA to be tested is related to the primer and probe used in the test.

[Precautions]

1.The detection results of this kit are for scientific research use only. Please read this instruction carefully before use.

2.In this kit, the amplification system consisting of the positive quality control, the supporting forward primer of positive control, reverse primer of positive control and the probe of positive control is mainly used to evaluate whether the reagent in the kit is valid.

3.To avoid cross contamination, the Reagent Preparation Area, Sample Addition Area and Detection Area should be separated.

4.A blank control without a template is used to determine whether there is a to-be-amplified nucleic acid contamination.

5.It is recommended to add samples in a special Sample Addition Area or biosafety cabinet to avoid reagent contamination caused by positive RNA.

6.All the to-be-tested sample or reagents should be treated as contagious substance. Please wear disposable gloves and lab-gown during the experiment process to protect the staff and avoid cross contamination.

7.Do not use expired product.

8.The kit is transported in the way of foam box and refrigerant for 7 days. The kit performance will not be affected if the temperature is not higher than 20 ℃.

[Approval Date and Revision Date of the Instruction Manual]:

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S00R01A / 1.3 2020/4/11 S00R01A / 1.4 2020/11/3 S00R01A / 1.5 2022/5/15

[Sign Interpretation]

IVD	The product is used in vitro, please don't swallow it.		Please don't reuse it
	Validity		Please read the instruction book carefully before using
	Warning, please refer to the instruction in the annex		Manufacturer
	Temperature scope within which the product is reserved		Keep dry
LOT	Batch number	REF	Catalogue number
	Avoid overexposure to the sun		Don't use the product when the package is damaged
	Date of manufacture		Contains sufficient for <*n*> tests
	European union authorization representative		
CE	The product meets the basic requirements of European In Vitro Diagnostic Medical Devices Regulation (EU)2017/746		

For research use only, cannot be applied in treatment or diagnosis of other fields.

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