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

【Product Name】

RT-RAA Nucleic Acid Amplification Kit (Test Strip Method)

【Specification】

48 Tests/Kit、96 Tests/Kit

【Intended Use】

This kit is for scientific research purpose；it can be used to amplify different RNA fragments. The amplification result can be verified by Lateral Flow Test Strip.

【Kit Contents】

Component	Volume	48 Tests/Kit	96 Tests/Kit
Purified Water	1500 μL/Tube	1 Tube	1 Tube
Buffer V	1500 μL/Tube	1 Tube	2 Tubes
Magnesium Acetate Ⅰ	600 μL/Tube	1 Tube	1 Tube
RT-Reaction Unit(T)	12 T/Pack	4 Packs	8 Packs
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Notes：Self-supplied Materials: Nucleic Acid Extraction Kit, Test Strip, PBS or PBST Buffer.

【Detection Principle】

This kit adopts RAA (Recombinase Aided Amplification) to amplify target nucleic acid sequence，rapid detection can be carried out combined with Lateral Flow Test Strip principle.

RT-RAA is an isothermal nucleic acid amplification technique that synchronizes the reverse transcription process with the amplification process. In constant temperature (Normally 37～42℃, 37℃ is recommended), within 20-40 minutes, the target gene fragment can be amplified by using the sample RNA as the template. Primers and probe are added during the experiment. The probe labels are recommended to use FAM or FITC, and the downstream primer are labeled with biotin. Labels can also be done according to the user's needs, the test strip should be coated with the corresponding antibody to achieve color.

This Kit can be used in scientific research. To ensure the rapid, sensitive and effective amplification. 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.

【Storage and Expiry Date】

1. 12 months when stored at -20℃ in the dark and avoid repeated freeze-thaw.Reaction unit can be stored for 1 month after unsealing.
2. 1 month when stored at 4℃ in the dark.

【Applicable Instrument】

1. It is recommended to use Sample Pretreatment System RAA-B6108 manufactured by Wuxi Qitian Biological Science Instrument Co., Ltd.
2. Thermostatl instruments(water bath).
3. Mini centrifuge.

【Detection Procedures】

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. Reagent Preparation---【Reagent Preparation Area】

(1) Sample Pretreatment System RAA-B6108 is started 30 minutes before the detection(or tun on the thermostat instrument(water bath) and set the temperature to 37℃).

(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 V. 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

Volume of 1×Reaction Mix

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

Notes：① n means the addition volume of RNA template，if the template concentration is high, the recommended addition volume of template is 1μL.
② Purified water can be used as negative control.
③ If 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 wastage).

(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.

3. 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 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 to prevent pollution caused by aerosol formation. Add one sample, close the lid, and then add the next sample, the sequence of addition is: negative sample/negative control, RNA to be tested, positive sample/positive control, mix well and centrifuge to collect.

（**Note**：this step cannot finished with Votex Oscillator, it is recommended to use supporting Sample Pretreatment System RAA-B6108 manufactured by Wuxi Qitian Biological Science Instrument Co., Ltd.）

4. Amplification Detection---【Amplification Detection Area】

4.1 Operation steps with RAA-B6108

（1）Symmetrically place the reaction tubes with sample RNA included into the RAA-B6108，Short press the "pretreatment key" and react for 7 minutes;

（2）After the reaction finished, the reaction unit was placed at 37 ℃ for 20-40 minutes.

4.2 Operation steps without RAA-B6108

（1）Put the reaction tube with the sample RNA included into the min centrifuge for short centrifugation;

（2）The reaction tube was fully mixed by hand and centrifuged briefly in the mini centrifuge(Note: Results may be affected if not fully mixed);

（3）Place the reaction tube in a 37 ℃ thermostat water bath for 7 minutes;

（4）Repeat step (2) after step(3)；

（5）The reaction unit was placed in the 37 ℃ thermostat water bath for 20-40 minutes.

5. Product Analysis---【Product Analysis Area】

The amplification products were diluted 10 times with PBS or PBST buffer，and detected with corresponding labeled strips。

6. Results Analysis

(1) Negative: Only C Line.

(2) Positive: Both T Line and C Line.

(3) Positive: Only T Line；

(4) C Line and T Line do not appear, the sample could be suspicious sample, the effectiveness of the test strip should be confirmed.

【Product Performance Indicators】

The plasmid with 10 copies/test can be stably amplified at 37℃; the LOD of the sample RNA to be tested is related to the primer and the probe used in the test.

【Notes】

1. The detection results of this kit are for scientific research only.Please read this instruction carefully before use.
2. The aerosol pollution of the amplification product can cause false positive easily；
3. To avoid cross contamination, the Reagent Preparation Area, Sample Addition Area, Product Analysis Area and Product Analysis Area should be separated.
4. 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.
5. Do not use expired product.
6. 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 ℃.
7. A blank control without template shall be set in the experiment to confirm whether the nucleic acid to be amplified is contaminated.

【Approval Date and Revision Date of the Instruction Manual】

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【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	Σ	Contains sufficient for <*n*> tests
EC REP	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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