RAA Nucleic Acid Amplification Kit
Instruction Manual

【Product Name】

RAA Nucleic Acid Amplification Kit

【Specification】

48 Tests/Kit, 96 Tests/Kit

【Intended Use】

This kit is for scientific research purpose；it can be used to amplify different DNA fragments. The amplification result can be verified by agarose gel electrophoresis.

【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
Reaction Unit(B)	12 T/Pack	4 Packs	8 Packs
Forward Primer of Positive Control(B)	25 μL/Tube	1 Tube	1 Tube
Reverse Primer of Positive Control(B)	25 μL/Tube	1 Tube	1 Tube
Positive Control(B)	25 μL/Tube	1 Tube	1 Tube
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Note： Self-supplied Materials: Nucleic Acid Extraction Kit, Phenol/chloroform (1:1), Reagent and equipment for agarose gel electrophoresis.

【Detection Principle】

This kit adopts RAA (Recombinase Aided Amplification) to amplify target nucleic acid sequence, the amplified products can be analyzed by agarose gel electrophoresis.

RAA is an isothermal nucleic acid amplification technology. In constant temperature (normally 37-42 ℃), within 20-40min，the sample DNA as template can be amplified.

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±5℃ in the dark and avoid repeated freeze-thaw.Reaction unit can be stored for 1 month after unsealing.
2. 1 month when stored at 2-8℃ 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. Thermostat 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）	2μL
Reverse Primer（10μM）	2μL
Purified Water	(16-n) μL
Total	(45-n) μL

Note： ① n means the addition volume of DNA 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 DNA 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 5 μL magnesium acetate Ⅰ in the tube lid，then add n μL negative control or DNA 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, DNA to be tested, positive sample/positive control, mix well and centrifuge to collect.

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

4.1 Ooperation steps with RAA-B6108

（1）Symmetrically place the reaction tubes with sample DNA included into the RAA-B6108，Short press the "pretreatment key" and react for 4 minutes;
（2）After the reaction finished, the reaction unit was placed at 37 ℃ for 40 minutes.

4.2 Operation steps without RAA-B6108

（1）Put the reaction tube with the sample DNA included into the mini 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 4 minutes;
（4）Repeat step (2) after step(3)；
（5）The reaction unit was placed in the 37 ℃ thermostat water bath for 40minutes.

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

（1）After the end of the reaction, take out the reaction tubs, add 50μL Phenol/chloroform (1:1) to each reaction tube, tightly close the tube lid, mix well(Note：this step can finished with Votex Oscillator), centrifuge to separate the liquid in the mini centrifuge.

（2）Pipette10 μL supernatant as amplification product, conduct agarose gel electrophoresis(The gel concentration is normally 1.5 ~ 2%) to detect.

【Product Performance Indicators】

The plasmid with 1000 copies/test can be stably amplified at 37℃; the LOD of the sample DNA to be tested is related to the primer 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. 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 Product Analysis 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 DNA.
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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S00000A / 1.6 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	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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