Nucleic Acid Extraction and Purification

Instructions for Use

【PRODUCT NAME】

Spin Column RNA kit

【PRODUCT MODEL】

Silica Gel Membrane Adsorption

【SIZE】

32 tests/kit

【INTENDED USE】

This product is intended for the extraction, enrichment and purification of nucleic acids. The processed products are used for clinical in vitro detection. (This kit is applicable to serum, plasma, whole blood, nose swab, pharynx swab, nasopharynx swab and urogenital swab samples).

【PRINCIPLE OF DETECTION】

This product is based on silica membrane specific adsorption principle to achieve nucleic acid separation.

【PRODUCT CONTENTS】

Table 1 Main Components

Number	Components	Amount	Amount in 1 reaction	Ingredient
1	Splitting Combined Buffer B	12.8mL/bottle	400μL	Tris, GuHCl, SDS
2	Wash Buffer W1B	9.6mL/bottle	500μL	Tris, GuHCl
3	Wash Buffer W2	6.4mL/bottle	500μL×2 次	Tris, NaCl
4	Elution Buffer	3.5mL/bottle	100μL	Tris
5	Carrier RNA	128μL/tube	4μL	PolyA
6	Protease K	640μL/tube	20μL	Protease K

Note: 1. Do not mix the components from different batches. Users need to prepare anhydrous ethanol and isopropanol;

2. Carrier RNA is made of freeze-dried powder, which needs to be dissolved with 128μL eluent buffer before use.

【STORAGE & SHELF LIFE】

The reagents are stable for 12 months while stored at -15 ℃~-25 ℃. See label for production date and expiration date.

【SAMPLING & HANDING】

Whole blood: Take 5mL of venous blood from patients with vacuum blood collection (anticoagulant EDTA) and mix it upside down for at least 8 times immediately.

Serum: Use disposable syringe or vacuum blood collection vessel without anticoagulant to extract 5mL of patient's venous blood, place it in sterile disposable test tube or vacuum blood collection vessel at room temperature, and take the separated serum for examination.

Plasma: Take 5mL of venous blood from patients with vacuum blood collection vessel (anticoagulant: heparin, EDTA, citrate, ACD), mix it upside down immediately for at least 8 times, centrifugate it at 4000rpm for 5 minutes, and take the separated plasma for examination.

Serum and plasma samples can be stored for 48 hours at 2-8 ℃ and frozen for a long time at -20℃. Repeated freezing and thawing of samples shall be avoided. The separated serum and plasma samples need to be preserved in an ice bottle during transportation. The whole blood sample can be kept for 3 hours at 2-8℃.

Nasal swab: Parallel to the upper palate, insert the swab into the nasal cavity, place it for 2-3 seconds to absorb the secretion; both sides of the nasal cavity should be sampled.

Pharyngeal swab: Go deep into the mouth, wipe the posterior pharyngeal wall and tonsils on both sides with moderate force to avoid touching the tongue.

Nasopharynx swab: Slowly extend the nasopharynx swab into the nostril until the back of the nasopharynx (Note: do not use force), turn it gently, and stay for about 20-30 seconds, then take it out quickly.

Urogenital swab: Both male and female urethra and cervix need to be inserted into urethra or cervix 2-3cm place and forced to rotate 1-2 circles for inspection, to avoid being contaminated by vaginal secretion swab.

The collected nasopharynx swab, pharynx swab, nasopharynx swab and urogenital swab were quickly put into a sterile test tube containing 1ml physiological saline, and the tube cover was tightened and sealed to prevent drying. It can be kept for 48 hours at 2-8℃ and frozen for a long time at -70℃.

【PROTOCOL】

1. Reagent Preparation:

In the first unsealed use, 6.6ml absolute ethanol was added into the cleaning buffer w1b reagent bottle; 26.4ml absolute ethanol was added into the cleaning buffer W2 reagent bottle; 128 μ l eluent buffer was added into carrier RNA, all of which were fully mixed. The dissolved carrier RNA was stored at - 20 ℃ for 2 months.

2. Sample Processing

The whole blood, serum and plasma samples do not need to be processed.

Swab sample: shake well and rinse (if it needs to be preserved for a long time, transfer the rinse solution to a 1.5ml centrifuge tube, and store the rinse solution at - 20 ℃ and - 70 ℃ for a long time).

3. Usage Method

3.1 Add 400μL of cleavage binding buffer B, 4μL of carrier RNA, 200μL of sample and 20μL of protease K into 1.5mL centrifuge tube in turn, vortex and shake for 5 seconds, centrifuge the tube at low speed for several seconds, and hold it in a 70℃ metal bath for 10 minutes.

3.2 Add 500μL isopropanol, vortex oscillate for 5 seconds, and centrifuge the centrifuge tube at low speed for several seconds.

3.3 Add 550μL of the above sample cracking solution to the adsorption column, centrifugate 8000g for 1 minute, and discard the filtrate; Repeat this step one more time.

3.4 Add 500μL cleaning buffer W1B into the adsorption column, centrifugate 8000g for 1 minute, and discard the filtrate.

3.5 Add 500μL cleaning buffer W2 into the adsorption column, centrifugate 8000g for 1 minute, discard the filtrate; Repeat this step one more time.

3.6 Centrifuge the column at 13000g for 10 seconds, and place the column in a new 1.5mL centrifuge tube.

3.7 Add 100μL of elution buffer solution preheated at 70℃ in the center of the adsorption column, and let it stand for 1 minute at room temperature.

3.8 Centrifuge the column at 8000g for 1 min. the filtrate is the extracted nucleic acid, which can be directly detected by PCR or stored at -20℃.

【ASSAY EXPLAINATION】

Detection of RNA purity by absorbance ratio OD260/280;

The ratio is less than 1.4, indicating that there are more protein impurities.

【Limitations of Test Methods】

RNA cannot be extracted from dried blood points or blood clots.

【PERFORMANCE SPECIFICATIONS】

1. Purity of nucleic acid: absorbance ratio OD 260/280 ≥ 1.4;

2. Precision: with PCR amplification reagent, CV of CT value is ≤ 5% (n = 5).

【ATTENTIONS】

1. Please read the instructions of the kit carefully before the experiment, and operate in strict accordance with the instructions.

2. After adding anhydrous ethanol to the cleaning buffer w1b and W2, it is necessary to mix them well or else it will affect the output and the repeatability of the experiment; Please tighten the cover after the experiment to prevent ethanol

from volatilizing.

3. The purified nucleic acid is suitable for PCR, Southern blotting, RAPD, AFLP and other molecular biological experiments

4. RNA samples should be collected in sterile plastic containers without RNase as much as possible; non disposable glass or plastic containers should be treated in advance to ensure no RNase contamination.

5. The operation steps must be carried out in the biosafety cabinet or other basic protective facilities.

6. See Table 2 for possible problems and handling methods in the process of nucleic acid extraction and detection.

Table 2 Possible problems and handling methods of sample nucleic acid extraction and detection

Issue	Possible Causes	Proposal
Low RNA quantity	RNA degradation	Ensure the reasonable storage and transportation of samples; the samples after acquisition shall be tested immediately.
	Crystallization and precipitation of reagent components	The kit should be stored at room temperature (15-30℃); if the reagent component is found to be crystallized, it should be dissolved in a 70℃ constant temperature metal bath. Do not heat to boiling, do not heat in a confined space.
Poor RNA detection	Sample usage or PCR template dosage too much or too little; Degradation of purified RNA;	Appropriate adjustment of sample usage or PCR template addition; The purified RNA should be detected as soon as possible.

【General Information】

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