RNA Extraction AND Purification From Whole Blood without Red cell lysis

1. Product Name

Nucleic Acid Extraction and purification reagent

2. Package Specifications

24 Test/Kit　48Test/Kit

3. Intended Use

It is used for the extraction, enrichment, and purification of nucleic acids. The processed product is used for clinical in vitro testing. The product composition does not contain antigens, antibodies, or probe components that can specifically bind to samples.

4. principle

This kit is suitable for extracting total RNA from fresh or frozen whole blood samples (blood samples treated with anticoagulants such as citrate, EDTA, or heparin). It adopts nano-magnetic beads that specifically bind to RNA and a unique buffer system. The silica hydroxyl nano-magnetic beads can efficiently and specifically adsorb RNA, which can maximize the removal of impurity proteins and other substances. The extracted RNA has high purity and stable quality, without protein and other impurity contamination, and can be directly used in downstream experiments.

5. Composition

Item No.	271602-2F24	271602-2F48	
Kit Specification	24 Tests	48 Tests	Storage at 15-30℃
Buffer Lysis	1 Test/Strip × 24 Strips	1 Test/Strip × 48 Strips	
Buffer WA			
RNA Washing Solution			
Magnetic Beads (50mg/mL)			
DEPC Elution Buffer	5mL	5mL	
Magnetic Sleeves	3 Packs (2 Pieces/Pack)	6 Packs (2 Pieces/Pack)	
Instruction Manual	1 Copy	1 Copy	

6. Storage Precations

① Transport and storage at room temperature (15-30℃)

② The validity period of the kit is 12 months,and it should be used within the validity period.

③ 2mL centrifuge tubes need to be prepared by yourself , sterilized, and used for eluting RNA.

④ DTT needs to be prepared by yourself or purchased from the manufacture (Cat No.a-1064).

⑤ Buffer solution PBS and DNase I(2U/μL）(RNase-free molecular grade) need to be prepared by yourself or purchased from the manufacturer (Cat No. 1416026).

7. Precautions for Preventing RNase Contamination

To prevent contamination by exogenous RNase during RNA extraction, the following measures must be taken:

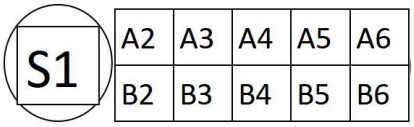
1. Operate with disposable clean gloves and masks to prevent RNase contamination from the skin, saliva, and laboratory supplies.
2. Use sterile and RNase-free plastic products and tips.
3. If glassware is used, it must be soaked in 0.1% DEPC water at 37℃ for 12 hours, then autoclaved at 121℃ for 30 minutes, dried, and then used.
4. For the preparation of corresponding reagents, 0.1% DEPC water autoclaved at 121℃ must be used.
5. Add 0.15g of DTT to 1mL of DEPC treated water for dilution, and mix well to obtain a 1mol/L DTT solution. It is recommended to prepare the DTT solution freshly before use. If the prepared DTT solution is not used up in the current operation, it can be stored at 2-8℃ for 14 days. For long-term storage, store it at -20℃ for 1 year, avoid repeated freezing and thawing, and the number of freeze-thaw cycles should not exceed 5 times. Undissolved DTT powder can be stored at -20℃ for a long time.

8. Operation Method

8.1 Things before your starts

1．Take 10 μL of RNase-free DNase I (2U/μL) (prepared by yourself) and add it to the reagent strip well A2.

2．According to the number of samples N, prepare N 2mL centrifuge tubes, add 70-105 μL of DEPC elution buffer, and set aside (70 μL of elution buffer is recommended for whole blood, and 90-105 μL for bone marrow blood).



Pic：Reagent strip

8.2 Operation Steps

(1) As shown in the figure: Take the reagent strip out of the kit, invert it up and down to mix the magnetic beads well, and gently flick to make the liquid flow to the bottom of the tube to avoid affecting the tearing of the sealing film.

(2) Add 1 mL of whole blood or 300 μL of Bone marrow blood and 60 μL of DTT solution to the S1 well of the reagent strip.

(3) Put the reagent strip into the card slot of the MDX-12 nucleic acid extractor, and place the 2mL centrifuge tube containing DEPC elution buffer at the S7 position of the instrument.

(4) Insert the sleeves and run the nucleic acid extractor program which mentioned as bellow (rotational speed: 121 rpm, in-liquid stirring).

Protocol used in this experiment:

Step	Well Position	Pause	Step Name	Waiting Time	Mixing Time Min:Sec	Absorption Time Min:Sec	Vol µl	Mixing Speed	Heating Setting	Temperature (℃)
1	B2	×	Magnet Transfer	0:0	0:00	40	800	Fast	Off	0
2	S1	×	Lysis	0:0	20:00	120	4060	Medium	Off	0
3	B2	×	Washing 1	0:0	1:00	40	800	Fast	Off	0
4	B3	×	Washing 2	0:0	1:00	40	800	Fast	Off	0
5	B4	×	Washing 3	0:0	1:00	40	800	Fast	Off	0
6	B5	×	Washing 4	0:0	1:00	40	800	Fast	Off	0
7	B6	×	Washing 5	0:0	1:00	40	800	Fast	Off	0
8	A2	×	Removal	2:0	10:00	40	200	Slow	Off	0
9	A3	×	Washing 6	0:0	1:00	40	800	Fast	Off	0
10	A4	×	Washing 7	0:0	1:00	40	800	Fast	Off	0
11	A5	×	Washing 8	0:0	1:00	40	800	Fast	Off	0
12	A6	×	Washing 9	0:0	1:00	40	800	Fast	Off	0
13	S7	×	Elution	1:0	5:00	60	120	Slow	On	50
14	A6	×	Discarding	0:0	0:20	0	800	Fast	Off	0

(5) After the program is completed, take out the 2mL centrifuge tube and place it at -80℃ or proceed to the next experiment (if there are magnetic beads remaining, they can be removed by centrifugation at 12000 rpm or magnetic attraction). Other consumables should be disposed of as medical device waste after being taken out of the instrument.

Note 1: For some bone marrow samples with excessively high cell content, magnetic bead residue is inevitable. For centrifuge tubes with subsequent magnetic bead residue, use a pipette to add 20-100 µL of DEPC water (it is recommended to measure with an ultra-micro spectrophotometer and dilute by gradually increasing the amount of DEPC water), vortex and mix well, centrifuge at 12000 rpm for 1 minute, and transfer to a new 1.5mL centrifuge tube for later use.

Note 2: For bone marrow samples, it is preferred to use a pipette with a range of 200 µL for sample addition. For bone marrow blood with obvious resistance during pipetting, the cell count is too high, which will affect the subsequent DNase I digestion. Adjust the pipette range to 50 µL, pipette 50 µL of the sample into a 1.5mL centrifuge tube, add 250 µL of PBS buffer solution or 250 µL of normal saline, mix well, and transfer all the samples to the S1 well.

Note 3： Note: The concentration of DNase I (Deoxyribonuclease I) varies across different manufacturers. Please read the instruction carefully. The total amount of enzyme to be added to each Well A2 is 20 U, and the volume can be converted based on different concentrations:

EXAMPLE：

① For DNase I with a concentration of 5 U/µL, only 4 µL needs to be added to Well A2;

② For DNase I with a concentration of 1 U/µL, only 20 µL needs to be added to Well A2.

9. Determination of RNA Purity and Concentration

9.1 Integrity

The integrity of RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: 1.2% gel; 0.5×TBE buffer solution; 150V, 15 minutes). After electrophoresis, 70-80% of the cellular rRNA shows very obvious rRNA bands under ultraviolet light, with sizes of approximately 5kb and 2kb, corresponding to 28S and 18S rRNA respectively.

9.2 Purity

OD260/OD280 is an indicator for measuring the degree of protein contamination. For high-quality RNA, the OD260/OD280 value ranges from 1.8 to 2.1, and a ratio of 2.0 is a marker of high-quality RNA. The OD260/OD280 value is affected by the pH value of the solution used for determination. For the same RNA, assuming that the OD260/OD280 values measured in 10mM Tris and pH7.5 solution are 1.8 and 2.1 respectively, the values measured in aqueous solution may range from 1.5 to 1.9, but this does not mean that the RNA is impure.

9.3 Concentration

Take a certain amount of purified RNA, dilute it n times with RNase-free ddH2O, blank the spectrophotometer with RNase-free ddH2O, measure the OD260 and OD280 values of the diluent, and calculate the RNA concentration according to the following formula: Final Concentration (ng/μL) = (OD260) × (Dilution Factor n) × 40

10. Basic Information

- Manufacturer: Guangzhou Surbiopure Biotechnology Co., Ltd.
- Address: Room 402, No. 16 Lianpu Street, Huangpu District, Guangzhou
- Service Hotline: 020-84783894
- Website: http://www.surbiopure.com

11.Record-filing Information

Yue Sui Xie Bei 20250359 (Record-filing No. of Medical Device in Guangzhou, Guangdong Province)