WB Human Blood RNA Extraction Kit

Instruction Manual
Ver. 04.19.24
For Research Use Only

BRNA/WBB/25;
BRNA/WBB/50;
BRNA/WBB/100

25 preparations;
50 preparations;
100 preparations

Wobble Base BioResearch, Gujarat, INDIA

Transportation: Room temperature
Storage: Room temperature

For research use only

A. Introduction

WB Human Blood RNA Extraction Kit comprise an effective solution for quick, efficient and cost-effective small-scale preparation of high-quality total RNA from fresh, whole human blood. Whole blood should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate and heparin, can also be used. For optimal results, blood samples should be processed within a few hours of collection. It employs the patented Hybrid spin column and WBshredder technology that assist in isolating Total RNA from whole blood clinical samples in less than 50 minutes.

Our specially formulated Lysis Buffer ensures highly efficient lysis of samples. Our reagents are designed to deliver high-yield and pure RNA extracts, perfect for downstream applications like such as RT PCR experiments.

B. Principle:

This Spin column-based nucleic acid purification kit follows a solid phase extraction method to quickly purify RNA.The method relies on the fact that RNA will bind to the solid phase of a specialized membrane under certain conditions.

The stages of the method are lyse, bind, wash and elute, [1][2*] i.e.* lysis of cells, binding of RNA to a membrane, washing the RNA bound to the membrane and elution of the RNA.

To lyse, the RBC (Red Blood Cells) are first broken and debris removed followed by concentration of the WBC (White Blood Cells). The WBC cells are then broken open with a lysis procedure which breaks the cell membrane and the nucleus to release the RNA.

For binding, a buffer solution is then added to the sample along with ethanol. This forms the binding solution. The binding solution is transferred to a spin column and the column is put in a centrifuge. The centrifuge forces the binding solution through a membrane that is inside the spin column. The pH and salt concentration of the binding solution are optimal and hence the RNA binds to the membrane as the solution passes through.

To wash, the flow-through is removed and a wash buffer is added to the column. The column is put in a centrifuge again, forcing the wash buffer through the membrane. This removes any remaining impurities from the membrane, leaving only the RNA bound to the membrane.

To elute, the wash buffer is removed and an elution buffer is added to the column. The column is put in a centrifuge again, forcing the elution buffer through the membrane. The elution buffer removes the RNA from the membrane and the nucleic acid is collected from the bottom of the column.

References:

1. Matson, Robert S. (2008). Microarray Methods and Protocols. Boca Raton, Florida: CRC. pp. 27–29. ISBN 1420046659.
2. Kumar, Anil (2006). Genetic Engineering. New York: Nova Science Publishers. pp. 101–102. ISBN 159454753X.

C. Kit contents:

Components	Volume Per reaction	25 tests	50 tests	100 tests
RBC lysis buffer	12 ml	2 x 150 ml	3 x 200 ml	5 x 250 ml
WLT Buffer	600 µl	16.5 ml	33 ml	66 ml
WW1 Wash Buffer #	650 µl	9 ml	18 ml	35 ml
WPE Wash Buffer #	1 ml	5.5 ml	11 ml	22 ml
RNA Elution Buffer	50 µl	1.5 ml	3 ml	6 ml
WBshredder column with collection tube	1 pc	25 pcs	50 pcs	100 pcs
Column with collection tube	1 pc	25 pcs	50 pcs	100 pcs
Elution tube	1 pc	25 pcs	50 pcs	100 pcs

 # To be reconstituted

D. Reconstitution of buffers/reagents

Wash buffers are supplied as a concentrate. Working buffers need to be prepared before use. Please see table below.

Buffer / Reagent name	Procedure for 25 Reactions
WW1 Wash Buffer #	Add 9 ml 100% Ethanol*
WPE Wash Buffer #	Add 22 ml 100% Ethanol*

Buffer / Reagent name	Procedure for 50 Reactions
WW1 Wash Buffer #	Add 18 ml 100% Ethanol*
WPE Wash Buffer #	Add 44 ml 100% Ethanol*

Buffer / Reagent name	Procedure for 100 Reactions
WW1 Wash Buffer #	Add 35 ml 100% Ethanol*
WPE Wash Buffer #	Add 88 ml 100% Ethanol*

**Not supplied with this kit*

E. Storage and Kit Stability

- All other components and reagents can be stored at room temperature (15-25˚C) until the expiration date printed on the kit label.
- For extended storage or storage in dry conditions (humidity < 40%), store the columns at 2-8 ˚C to maintain efficacy & performance.

F. Material and instruments required

- Microcentrifuge capable of at least 14,000 g centrifugation
- Vortex with speed of at least 2,500 rpm
- Absolute ethanol
- Single-channel pipettors (5 µL–1000 µL)
- Nuclease-free, aerosol-preventive tips
- Powder-free gloves (disposable)
- Ethanol (96 – 100%)

[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.]

G. Product Use Limitations

- All reagents may exclusively be used in molecular biology grade RNA related applications.
- The product is to be used by personnel specially instructed and trained in molecular biology experiments.
- Strict compliance with the user manual is required for optimal results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective, disposable, powder-free gloves, laboratory coat and eye protection gear when handling specimens and kit components.
- Avoid microbial and nuclease (DNAse/RNase) contamination of the specimens and the components of the kit.
- Always use DNAse/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Discard sample and assay waste according to your local safety regulations.
- **For Query:** support@wobblebase.in ; +91 6353339602

H. Important Notes:

- All purification steps should be carried out at room temperature.
- All centrifugations should be carried out in a table-top micro-centrifuge at >1000 x g (8000-14000 rpm, depending on the rotor type).

I. Safety Precautions

Lysis Buffer and Wash buffer WW1 contain a component that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink or smoke when using this product. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes.

J. Adjustment of sample volume:

- If sample volume to be used is more, scale up the buffer volumes accordingly.

K. Procedure:

1. Mix 1.5 ml of human whole blood with 7 ml of RBC lysis Buffer in an appropriately sized tube (not provided).
   Note: Use an appropriate amount of whole blood. Up to 1.5 ml of healthy blood (Typically 4000–7000 leukocytes per microliter) can be processed.

Reduce amount appropriately if blood with elevated numbers of leukocytes is used.

2. Incubate for 10–15 min on ice. Mix by vortexing briefly 2 times during incubation.
   Note: Use of ice is essential for satisfactory extraction of RNA
3. Centrifuge at 1600 rpm for 10 min at 4°C and completely remove and discard supernatant.
4. Add 5 ml RBC lysis Buffer to the cell pellet. Resuspend cells by vortexing briefly.
5. Centrifuge at 1600 rpm for 10 min at 4°C and completely remove and discard supernatant using the pipette.
   Caution: Centrifuge temperature >4°C can compromise with the quality and quantity of the extracted RNA.
   Note: Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the spin column, resulting in lower yield.
6. Add 6 µl ß-Mercaptoethanol (Not provided) and 600 µl WLT Buffer to pelleted leukocytes. Vortex or pipet to mix.
7. Pipet the lysate carefully and directly into a WBshredder spin column placed on a 2 ml collection tube (provided) using the pipette and centrifuge for 2 min at maximum speed to homogenize. Discard WBshredder spin column and save the homogenized lysate.
8. Add 600 µl of 70% ethanol to the homogenized lysate collected after passing through the WBshredder spin column and mix by pipetting. Do not centrifuge.
9. Carefully pipet the sample now including any precipitate which may have formed, into a fresh purification spin column placed on a 2 ml collection tube (provided) without moistening the rim. Centrifuge for 50 s at 10,000 rpm.
   Note: Maximum loading volume is 700 µl. If the volume of the sample exceeds 700 µl, successively load aliquots onto the spin column (one after the other to add the entire volume into the column) and centrifuge each time as above to aloe binding of the RNA to the membrane.
10. Discard flow-through. Retain the used collection tube.
11. Transfer the spin column onto the previously used 2 ml collection tube. Apply 650 µl WW1 Buffer (Ethanol added) to the spin column and centrifuge for 50 s at 10,000 rpm to wash.
12. Discard flow-through. Retain the used collection tube.
13. Place spin column on to the used 2 ml collection tube. Pipet 500 µl of WPE Buffer (Ethanol added) into the spin column and centrifuge for 50 s at 10,000 rpm.
14. Discard flow-through. Retain the collection tube.
15. Carefully open the spin column and add (again) 500 µl of WPE Buffer (Ethanol added). Close the cap and centrifuge for 50 s at 10,000 rpm.
16. Discard flow-through. Retain the collection tube.
17. Place the spin column on the used 2 ml collection tube and centrifuge the blank spin column at full speed 12,000 rpm for 3 min without adding any solution into it (Empty spin). Discard the collection tube.
18. Transfer spin column now into a fresh, sterile 1.5 ml micro centrifuge tube (provided) and pipet 30–50 µl of RNA-Elution buffer (provided) directly onto the column membrane. Take care to drop the RNA Elution buffer right in the middle of the column membrane.
    Note: Do not substitute the RNA Elution buffer with any other solution as it will compromise with the quality of the extracted RNA.
19. Incubate for 2 min at room temperature and centrifuge for 1 min at 10,000 rpm to elute. Repeat step 18 if necessary with additional elution buffer for greater recovery.
    Note: Repeat elution dilutes the extracted RNA solution while increasing the total yield.
    Note: At all stages, 4^0C centrifugal conditions/ice (whichever applicable) is essential for extraction of satisfactory quality and quantity of RNA.
    Discard the purification column. Centrifuge tube now contains the eluted RNA. Either use the directly in PCR or store at -20°C or -80°C (for long ter storage) for later analysis.

L. Recommendation for Real-time PCR:
Use 5 - 10µl of elute

M. Quality Control

The WB Human Blood RNA Extraction Kit is tested on a lot-to-lot basis according to Wobble Base BioResearch quality management system. For this, total RNA is isolated from fresh whole human blood and tested against predetermined specifications to ensure consistent product quality.

N. Symbols used on product and labelling

Catalogue number

Amount of sample preparations

Lot number


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse


Manufacturer

Manufactured by

WOBBLE BASE BIORESEARCH PRIVATE LIMITED, Gujarat, INDIA

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