# WB Human Blood DNA Extraction Kit

Instruction Manual
Ver. 04.16.24
For Research Use Only

BDNA/WBB/25;
BDNA/WBB/50;
BDNA/WBB/100

25 preparations;
50 preparations;
100 preparations

Wobble Base BioResearch, Gujarat, INDIA

Transportation: Room temperature
Storage: Room temperature[1]

[1] *Digestion reagent: Transportation at room temperature, storage at -20ºC before and after reconstituted.*

## A. Introduction

WB Human Blood DNA Extraction Reagents comprise an effective solution for quick and efficient genomic DNA extraction from various types of human clinical sample. It employs the patented Hybrid spin column technology that assist in isolating genomic DNA from whole blood and other types of clinical samples in less than 30 minutes, without the need for homogenization!

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

## B. Principle:

Spin column-based nucleic acid purification is a solid phase extraction method to quickly purify nucleic acids. This method relies on the fact that nucleic acid will bind to the solid phase of silica under certain conditions.

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

To lyse, the cells of a sample are broken open with a lysis procedure which breaks the cell membrane and the nucleus to release the nucleic acid.

For binding, a buffer solution is then added to the sample along with ethanol or isopropanol. 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 silica gel membrane that is inside the spin column. When the pH and salt concentration of the binding solution are optimal, the nucleic acid bind to the silica gel 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 nucleic acid bound to the silica gel.

To elute, the wash buffer is removed and an elution buffer (or simply water) 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 nucleic acid from the membrane and the nucleic acid is collected from the bottom of the column.

**References:**

*Matson, Robert S. (2008). Microarray Methods and Protocols. Boca Raton, Florida: CRC. pp. 27–29. ISBN 1420046659.*

*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 |
|---|---|---|---|---|
| Digestion Reagent solute# | 2.2 ml from reconstitution solution | Desiccated powder | Desiccated powder | Desiccated powder |
| Reconstitution Solution for Digestion Reagent | 20 µl when reconstituted | 0.63 ml | 1.25 ml | 2.5 ml |
| Lysis Buffer | 200 µl | 5.5 ml | 11 ml | 22 ml |
| Wash Buffer 1# | 500 µl | 6.5 ml | 13 ml | 27.5 ml |
| Wash Buffer 2# | 500 µl | 5.5 ml | 11 ml | 22 ml |
| Elution Buffer | 100 µl | 3 ml | 6 ml | 11 ml |
| 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.
- Digestion Reagent solute should be stored at -20ºC on arrival. Add sterile Reconstitution Solution to the Digestion Reagent solute (see the bottle label for volume of Reconstitution Solution to be added) and vortex to ensure that the Digestion Reagent solute is completely dissolved. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. The Digestion Reagent solute mixture should be stored -20ºC.

| Buffer / Reagent name | Procedure for 25 Reactions |
|---|---|
| Digestion Reagent | Add 0.63 ml of Reconstitution Solution to the Digestion Reagent Solute; Mix well; Store **immediately at -20⁰C** |

| Wash Buffer 1 | Add 6.5 ml 100% Ethanol* |
|---|---|
| Wash Buffer 2 | Add 8.5 ml 100% Ethanol* |

| Buffer / Reagent name | Procedure for 50 Reactions |
|---|---|
| Digestion Reagent | Add 1.1 ml of Reconstitution Solution to the Digestion Reagent Solute; Mix well; Store **immediately at -20⁰C** |
| Wash Buffer 1 | Add 13 ml 100% Ethanol* |
| Wash Buffer 2 | Add 16.5 ml 100% Ethanol* |

| Buffer / Reagent name | Procedure for 100 Reactions |
|---|---|
| Digestion Reagent | Add 2.2 ml of Reconstitution Solution to the Digestion Reagent Solute; Mix well; Store **immediately at -20⁰C** |
| Wash Buffer 1 | Add 27.5 ml 100% Ethanol* |
| Wash Buffer 2 | Add 33 ml 100% Ethanol* |

**Not supplied with this kit*

## E. Storage and Kit Stability

- Digestion Reagent solute should be kept at –20°C after reconstitution. 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 condition (humidity < 40%), store the columns at 2-8 ˚C to maintain if 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
- Water bath/Heating block
- Absolute ethanol
- Single-channel pipettors (5 µL–1000 µL)
- Nuclease-free, aerosol-preventive tips
- Powder-free gloves (disposable)

[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 DNA/RNA 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 >12000 x g (8000-14000 rpm, depending on the rotor type).

**I. Safety Precautions**

Lysis Buffer and Wash buffer 1 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 your sample volume is less than 200µl, the sample volume should be adjusted with PBS (Phosphate Buffer Saline).
- If sample volume to be used is more, scale up the buffer volumes accordingly.

**Note: Set water bath or Dry bath to 56°C.**

**K. Procedure:**

1. Add 20 µL of Digestion reagent into 200 µL of human peripheral blood or amniotic fluid and mix by vortexing.
2. Add 200 µL of Lysis Buffer, mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3. Incubate the mixture at 56°C for 10-15 min while vortexing occasionally, using a water bath or a dry-bath until the cells lyse completely.
4. Add 200 µL of ethanol (100%) and mix well by pipetting.
5. Transfer the mixture to the spin column.
6. Centrifuge the Column at 8000 rpm for 1 min and discard the flow- through.
7. Insert the column into the Collection Tube and add 500 µl Wash Buffer-1 (Ethanol added) on to the column.
8. Centrifuge the column at 8000 rpm for 1 min and discard the flow-through.
9. Insert the column into the Collection tube and add 500 µl Wash Buffer-2 (Ethanol added).
10. Centrifuge the column at 14000 rpm for 1 min.
11. Discard the flow-through and place column in the collection tube.
12. Centrifuge the column at maximum speed (14000 rpm) for 2-3 min.
13. Place the Column into a clean 1.5 ml elution tube (supplied with the kit).
14. Add 80-100 µl elution Buffer to the centre of the column.
15. Incubate the column at room temperature for 1-2 min.
16. Centrifuge the column-elution tube pair at 8000 rpm for 1-2 min.
17. The elution tube now contains purified genomic DNA.
    - **Note**:
        - For maximum DNA yield, repeat the elution step (Step 13-17 above) with an additional 100 µl of Elution Buffer.
        - If more concentrated DNA is required or if DNA has been isolated from a small amount of starting material the volume of Elution Buffer added to the column can be reduced to 50-100 µL.
        - Lower volumes of Elution Buffer will result in lower overall yield of eluted DNA.
18. Discard the purification column. Use the purified DNA immediately for downstream applications or store at -20°C.

**L. Recommendation for Real-time PCR:**

Use 5 - 10µl of elute

**M. Quality Control**

The WB Human Blood DNA Extraction kit is tested on a lot-to-lot basis according to Wobble Base BioResearch quality management system. For this, genomic DNA is isolated from 200 µl of fresh whole human blood. The isolated DNA (5-15 µg with an A260/A280 ratio of 1.8–2.0) is quantified with a spectrophotometer and analysed for by electrophoresis.

**N. Symbols used on product and labelling**

s

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

WWW.WOBBLEBASE.IN | support@wobblebase.in | +91 63533 39602

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