WB Viral DNA Extraction Kit

Instruction Manual
Ver. 04.16.24
For Research Use Only

VDNA/WBB/25
VDNA/WBB/50
VDNA/WBB/100

25 preparations
50 preparations
100 preparations

Wobble Base BioResearch, Gujarat, INDIA

Transportation: Room temperature
Storage: Room temperature[1]

[1]Recovery Reagent and Flocculating reagent: Transportation at room temperature, storage at -20°C before and after reconstituted.

A. Introduction

WB Viral DNA Extraction Reagents comprise an effective solution for quick and efficient Viral DNA extraction from various types of human clinical sample like human blood, plasma, serum swab cell pellet, CSF, other body fluids and VTM. It employs the patented Hybrid spin column technology that assist in isolating viral DNA from plasma 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 and RT PCR directly.

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 free 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
Flocculating reagent	5.6 µl	152 µl	303 µl	616 µl
Recovery Reagent solute#	30 µl when reconstituted.	Desiccated powder	Desiccated powder	Desiccated powder
Reconstitution solution for Recovery reagent		0.9 ml	1.8 ml	3.5 ml
Viral Lysis Buffer	560 µl	15 ml	31 ml	62 ml
Wash Buffer#	1 ml	13.5 ml	27.5 ml	55 ml
Elution Buffer	50 µl	2 ml	3 ml	6 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.
- Flocculating reagent and Recovery Reagent solute should be stored at -20ºC on arrival. Add Reconstitution Solution to the Recovery Reagent solute (see the bottle label for volume of Reconstitution Solution to be added) and vortex to ensure that the Recovery Reagent solute is completely dissolved. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. The Recovery Reagent solute mixture should be stored -20ºC.

Buffer / Reagent name	Procedure for 25 Reactions
Recovery Reagent	Add 0.8 ml of Reconstitution Solution to the Recovery Reagent solute; Mix well; Store **immediately at -20ºC**
Wash Buffer	Add 13.5 ml 100% Ethanol*

Buffer / Reagent name	Procedure for 50 Reactions
Recovery Reagent	Add 1.6 ml of Reconstitution Solution to the Recovery Reagent solute; Mix well; Store **immediately at -20^{0}C**
Wash Buffer	Add 27.5 ml 100% Ethanol*

Buffer / Reagent name	Procedure for 100 Reactions
Recovery Reagent	Add 3.3 ml of Reconstitution Solution to the Recovery Reagent solute; Mix well; Store **immediately at -20^{0}C**
Wash Buffer	Add 55 ml 100% Ethanol*

*****_Not supplied with this kit_

E. Storage and Kit Stability

- The Recovery 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 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 150µl, 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.

Plasma/Serum/CSF/body liquids/fresh whole human blood: Gently mix well and transfer 150µl for the purification.

Viral carrier media containing Swab [VTM]: Vortex well with swab inside & safely discard the swab. Again, vortex well and transfer 150µl for the purification

Cervical Swabs stored in cytology media:

- Vortex well the tube with swab and carefully discard the swab.
- Centrifuge the tube at 8000 rpm for 5 min. using micropipette, carefully discard the supernatant without disturbing the pellet.
- Add 2 ml of sterile PBS buffer to the pellet and vortex well.
- Centrifuge at 10,000 rpm for 3 min and discard the supernatant.
- Add 2ml of sterile PBS buffer to the pellet and vortex well.
- Centrifuge at 10000 rpm for 2 min and discard the supernatant.
- Vortex well the pellet to dislodge, add 200µl of sterile PBS buffer and use 150µl for purification.

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

K. Procedure:

i. Add 5.6 µL of Flocculating reagent and 30 µL of Recovery buffer to 150 µL of Sample and mix by gentle vortexing.
ii. Add 560 µL of Lysis Buffer, mix thoroughly by vortexing or with the help of an auto-pipette to obtain a uniform suspension. Vortex and short spin.
iii. Incubate the sample at 56 ^{0}C for 15 minutes and vortex occasionally until the cells lyse. Short spin.
iv. Add 560 µL of ethanol (100%) and mix well by pipetting. Total volume now will be around 1276 µL.
v. Transfer 650 µl of the solution (approximately) to a spin column. Centrifuge the column at 12,000 rpm for 1 minute. Discard the flow - through. Insert the column into a collection tube. Add the remaining volume on to the spin column. Centrifuge the column at 12,000 rpm for 1 minute. Discard the flow - through.
vi. Insert the column into a collection tube and add 500 µl of Wash Buffer. Centrifuge the column at 12,000 rpm for 1 minute. Discard the flow-through. Place the column into a fresh collection tube.
vii. Add 500 µl of Wash Buffer. Centrifuge at 12000 rpm for 1 minute. Discard the flow-through.
viii. Place column on to the fresh collection tube. Centrifuge the column again (without any wash buffer) at maximum speed (14000 rpm) for 2-3 minutes.
ix. Place the Column on to a clean, sterile 1.5 ml elution tube (supplied). Add 50µl of pre-warmed (56°C) Elution Buffer to the centre of the column and incubate at room temperature for 1-2 minutes. Centrifuge the column at 14,000 rpm for 1-2 minutes. The elution tube now contains purified viral DNA.
Note:
    - *For maximum viral DNA yield, repeat the elution step (Spin-ix) with an additional 50 µl of Elution Buffer.*
    - *Lower volume of Elution Buffer will result in reduced total yield of eluted viral DNA.*
x. Discard the purification column. 1.5 ml tube now contains the eluted Viral DNA. Either use immediately for downstream applications or store at -20°or -80°C for later analysis.

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

M. Quality Control

The WB Viral DNA Extraction kit is tested on a lot-to-lot basis according to Wobble Base BioResearch quality management system, each lot of Viral DNA Extraction kit is 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

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

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