Nucleic Acid Extraction Kit (Magnetic Bead Method)

Date of Issue	Mar, 2022
Current Version	01
Language	English
Document ID	FZ-01.20.60.03.1656-01
Applicable instruments	Zybio EXM3000/3200, Zybio EXM6000/6200, TIANLONG NP968-C
Specimen Types	Whole blood sample with EDTA (K2/K3) and citrate anticoagulation.
Reagent Storage	2～8°C
Shelf-life Stability	12 months

Intended Use

This kit is used for the extraction, enrichment and purification of nucleic acid (DNA/RNA) in whole blood samples. The purified nucleic acid can be used in downstream molecular biology experiment.

Test Principles

The magnetic beads in the kit have specific polymeric groups of adsorbed nucleic acid (DNA/RNA) on the surface. In special conditions like hypersaline, cells or viruses in the samples lyse rapidly and release nucleic acids, which are specifically adsorbed by magnetic beads. Nucleic acids on the magnetic beads will be separated from the liquid phase when the magnetic separator is used. Residual impurities and inhibitors in the liquid phase are removed by washing. Finally, nucleic acids are eluded from the magnetic beads by changing the liquid phase conditions, so as to separate nucleic acid rapidly and efficiently.

Materials Provided

REF	Specification	Packing Size	Applicable Instruments
01.09.20.03.BP.10	WB-B	8 T/Kit	Zybio EXM3000/3200, TIANLONG NP968-C
01.09.20.03.BP.11	WB-B	16 T/Kit	
01.09.20.03.BP.12	WB-B	20 T/Kit	
01.09.20.03.BP.13	WB-B	32 T/Kit	
01.09.20.03.TP.05	WB-T	32 T/Kit	Zybio EXM6000/6200
01.09.20.03.TP.06	WB-T	96 T/Kit	

Main Components

Table 1 Main Components of WB-B

Constituents	WB-B				Main Components
	8 T/Kit	16 T/Kit	20 T/Kit	32 T/Kit	
Proteinase K	160 μL× 1 tube	320 μL× 1 tube	400 μL× 1 tube	640 μL× 1 tube	10mg/mL-35mg/mL Proteinase K
Nucleic Acid Extraction Reagent Prepackaged in 96-Well Plates	4 T× 2 plates	8 T× 2 plates	1 T× 20 plates	16 T× 2 plates	Lysis Solution, Magnetic Beads Solution, Washing Solution I, Washing Solution II, Elution Solution
8-strip magnetic tips comb	4 pcs	4 pcs	10 pcs	4 pcs	/

Table 2 Main Components of WB-T

Constituents	WB-T		Main Components
	32 T/Kit	96 T/Kit	
Proteinase K	640 μL×1 tube	960 μL×2 tubes	10mg/mL-35mg/mL Proteinase K
Lysis Solution	32 T×1 plate	96 T×1 plate	35%-40% Guanidine thiocyanate, Purified Water
Washing Solution I	32 T×1 plate	96 T×1 plate	55%-65% Anhydrous Ethanol, Purified Water
Washing Solution II	32 T×1 plate	96 T×1 plate	55%-65% Anhydrous Ethanol, Purified Water
Elution Solution	32 T×1 plate	96 T×1 plate	Purified Water
Magnetic Beads Solution	32 T×1 plate	96 T×1 plate	0.2%-0.4% Magnetic Beads

Warnings and Precautions

- Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation.
- Use sterile centrifuge tubes and filter-tips for sample preparation.
- All specimen to be tested should be considered as infectious substances and processed strictly in accordance with laboratory biosafety requirements. Sample should be prevented from freeze-thaw cycles, mixed well before use. Otherwise, the amount of extracted DNA/RNA extracted will decrease.
- The disposal of waste liquid should be in accordance with local laws and regulations.
- All components need to be mixed well before use. If there is a small amount of crystallization in solutions, reagent cannot be used until it is fully dissolved.
- ⚠ [Lysis Solution] contains guanidine thiocyanate. Please take care to protect yourself from skin or eye damage when using.
- Considering [Washing Solution] contains flammable components, please keep away from fire sources or other risk factors.
- After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution, and then cleaned with 75% ethanol or pure water. Finally, turn on UV light (if available) to disinfect working surfaces for 30 minutes.
- Check the integrity of the reagent before use. If the 96-well plate is found to have leakage, insufficient volume, no reagents in the wells, etc., please stop using such kit. Use a new kit for extraction.
- 96-well plates of WB-B specifications and 96-well plates of WB-T specification may have sealing film difficult to remove, partial film residual on the plate, plate deformation, etc., please stop using such kit. Use a new kit for extraction.

Storage & Stability

- The kit is stored at 2 ~ 8°C in dry, valid for 12 months.
- Once opened, 96-Well plates are disposable.

Sample Requirements

- Samples should be collected, transported, stored, and processed according to the relevant regulatory requirements.
- Sample types: Whole blood sample with EDTA (K2/K3) and citrate anticoagulation.

zybio CE IVD

Nucleic Acid Extraction Kit (Magnetic Bead Method)

- The collected samples can be used for nucleic acid extraction immediately, or stored at 2~8°C for less than 2 weeks, at -25°C ~-15°C for less than 3 months, full mixing before use, prevent from freeze-thaw cycles.
- The samples should be transported in sealed cooler box or styrofoam box with ice seal.
- The isolated products should be used for subsequent detection immediately, or stored at 2~8°C for no more than 48 hours, at -25°C ~-15°C for no more than 12 months.

Sample Preparation

This kit requires 200 μL of sample for a single determination. Place samples in a biological safety cabinet. If the sample is frozen, completely thaw it at room temperature before use.

Extraction Procedure

1. Extraction Procedure with EXM3000/3200, TIANLONG NP968-C (For WB-B)

Please follow EXM3000/3200 Nucleic Acid Isolation System User Manual, Nucleic Acid Extractor NP968-C User Manual for extraction setup.

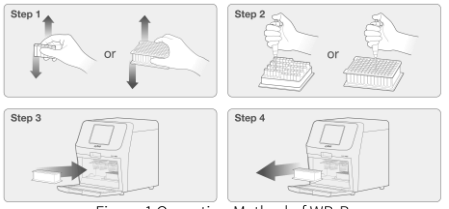

Figure 1 Operation Method of WB-B

1) Take out all the components in the kit. Mix the 96-well plate upside down so that the liquid adhering to the sealing film and the wall of the wells of the 96-well plate falls to the bottom of the plate. Let them stand for 3-5 minutes for use.
2) Carefully open the sealing film of the 96-well plate, and add 20 μL [Proteinase K] to the position A1~H1 and A7~H7 sequentially, then add 200 μL sample sequentially. Place the 96-well plate in the corresponding position of the instrument and insert the 8-strip magnetic tips comb. Close instrument door after finish loading.

Exception: *For WB-B-20, label facing the operator, carefully open the sealing film of the 6-well reagent cards, and add 20 μL [Proteinase K] and 200 μL sample to the position 1. Then put the reagent cards into the baseplate crosswise (as shown in Step 2), put the baseplate into the corresponding position of the instrument, and insert 8-strip magnetic tips comb. Close instrument door after finish loading. The next operations are the same.*

3) Turn on the nucleic acid isolation system, enter the page < program edit >, and set the isolation procedure according to Table 3.

Table 3 Running Program Setting

No.	Name	Position	Volume	Mixture Velocity	Mixing Time (min)	Absorption Magnetic Beads Time (sec)	Waiting Time (min)	Temperature (℃)
1	Move Magnetic	2	600	5	1.0	30	0.0	Closed
2	Lysis	1	500	3	10.0	60	0.0	Closed
3	Wash1	3	600	3	1.0	30	0.0	Closed
4	Wash2	4	600	3	1.0	30	0.0	Closed
5	Elution	6	85	3	6.0	180	0.0	85
6	end	1	300	1	0.0	0	0.0	Closed

Note: *The parameters of volume set according to the actual extraction effect, may not be the same with reagent volumes. Please carefully check that the 8-strip magnetic tips comb has been inserted into the appropriate position before the program runs.*

For EXM3000/3200 Nucleic Acid Isolation System: Turn on the nucleic acid Extraction system, select the <ZYBIO-WB-B-200>.

4) Click "Start" to run the extraction program. The process takes about 27 minutes.
5) Transfer the nucleic acid solution at the A6-H6 and A12-H12 positions of the 96-well plate to 1.5 mL centrifuge tube for further use (a small amount of beads can be removed by centrifuge or magnetic stand).

2. Extraction Procedure with EXM6000/6200 (For WB-T)

Please follow EXM6000/6200 Nucleic Acid Isolation System User Manual for extraction setup.

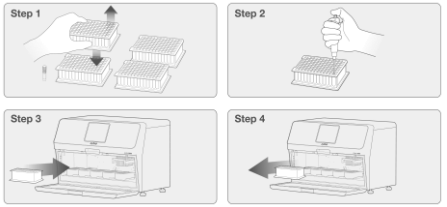

Figure 2 Operation Method of WB-T

1) Take out all the components in the kit. Mix the 96-well plate upside down so that the liquid adhering to the sealing film and the wall of the wells of the 96-well plate falls to the bottom of the plate. Let them stand for 3-5 minutes for use.
2) Carefully open the sealing film of the 96-well plate. According the number of the samples, add 20 μL [Proteinase K] sequentially to the 96-well plate of [Lysis Solution], then add 200 μL sample sequentially.
3) Put the 96-well plate of [Lysis Solution] into the NO.1 position of the instrument. Put the 96-well plate of [Magnetic Beads Solution] into the NO.2 position of the instrument and steadily insert the 96-strip magnetic tips comb. Put the 96-well plate of [Washing Solution I] into the NO.3 position of the instrument. Put the 96-well plate of [Washing Solution II] into the NO.4 position of the instrument. Put the 96-well plate of [Elution Solution] into the NO.5 position of the instrument. Close instrument door after finish loading.

Note: *Please carefully check that the 96-strip magnetic tips comb has been inserted into the appropriate position before the program runs.*

4) Turn on the nucleic acid extraction system, select the <ZYBIO-WB-T-200> program.
5) Click "Start" to run the extraction program. The process takes about 28 minutes.
6) Take out the 96-well plate at the NO.5 position of the instrument, and the nucleic acid solution can be used directly for further use, or transferred to 1.5 mL centrifuge tube for use (a small amount of beads can be removed by centrifuge or magnetic stand).

Limitations

- All user, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform

Nucleic Acid Extraction Kit (Magnetic Bead Method)

the test and interpret the results prior to performing the test independently.

- Performance of this kit may be affected by the source of specimens, transportation of specimens, storage conditions, specimen types, and other factors that have not been evaluated.

Performance Characteristics

- Yield: The total amount of nucleic acid isolated from 200 μL whole blood sample is ⩾4 μg.
- Purity: $OD_{260/280}$ >1.7.

References

1. Tang Y J，Zou J，Ma C，et al. Highly Sensitive and Rapid Detection of Pseudomonas aeruginosa Based on Magnetic Enrichment and Magnetic Separation. Theranostics,2013, 3(2):85-92.
2. Song Y, Feldman C, et al. A reliable and effective method of DNA isolation from old human blood paper cards [J]. Spring erPlus, 2013, 2(1): 1-7.
3. Li J M. Real-time Fluorescent PCR Technology [M]. Beijing: People's Military Medical Press, 2007.

Symbol Interpretation

Symbol	Title and Description	Symbol	Title and Description
IVD	In vitro diagnostic medical device	LOT	Batch code
	Consult instructions for use		Use-by date
CE	CE marking of conformity		Manufacturer
EC REP	Authorized Representative in the European Community		Temperature limit
REF	Catalogue number		Do not re-use
	Date of Manufacture		Warning

Zybio Inc.
Floor 1 to Floor 5, Building 30, No.6 of Taikang Road, Block C of Jianqiao Industrial Park, Dadukou District, 400082 Chongqing, PEOPLE'S REPUBLIC OF CHINA.
Web: www.zybio.com
E-mail: info@zybio.com
Tel: +86 (0)23 6895 9999
Fax: +86 (0)23 6869 9779

EC REP Lotus NL B.V.
Koningin Julianaplein 10, 1e Verd, 2595AA,
The Hague, Netherlands

zybio
CE IVD