Nucleic Acid Extraction Kit (Magnetic Bead Method)

CE IVD

Product name

Nucleic Acid Extraction Kit (Magnetic Bead Method)

Product code

BF-A, BF-B, BF-T

Packing specifications

Catalog No.	Package size	Catalog No.	Package size
BF-A-32	32 T/Kit	BF-B-20	20 T/Kit
BF-A-96	96 T/Kit	BF-B-32	32 T/Kit
BF-B-8	8 T/Kit	BF-T-32	32 T/Kit
BF-B-16	16 T/Kit	BF-T-96	96 T/Kit

Intended use

For the extraction, enrichment and purification of nucleic acid (DNA) in samples to be tested. Its processed products are for clinical in vitro diagnostics.

Test principle

The magnetic beads in the kit have specific polymeric groups of adsorbed nucleic acid (DNA) on the surface. In special conditions like high temperature and hypersaline, cells, viruses or bacteria 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.

Main components

Table 1 Main components of BF-A

Components of Kit	BF-A		Note
	32 T/Kit	96 T/Kit	
Pre-treatment Solution	3.2 mL× 1 bottle	9.6 mL× 1 bottle	Applicable to manual operation or semi-automatic nucleic acid extraction system.
Extraction Reagent I	16 mL× 1 bottle	48 mL× 1 bottle	
Extraction Reagent II	19.2 mL× 1 bottle	57.6 mL× 1 bottle	
Elution Buffer	6.4 mL× 1 bottle	19.2 mL× 1 bottle	
Magnetic Beads Solution	128 μL× 1 tube	384 μL× 1 tube	
Proteinase K	480 μL× 1 tube	1440 μL× 1 tube	
Instrument System Solution	/	19.2 mL× 1 bottle	

Table 2 Main components of BF-B

Components of Kit	8 T/Kit	16T/Kit	20T/Kit	32T/Kit	Note
Pre-treatment Solution	0.8 mL× 1 bottle	1.6 mL× 1 bottle	2.0 mL× 1 bottle	3.2 mL× 1 bottle	Applicable to semi-automatic nucleic acid extraction system.
Proteinase K	120 μL× 1 tube	240 μL× 1 tube	300 μL× 1 tube	480 μL× 1 tube	
96-Well Plates Prepackaged Nucleic Acid Extraction Reagent	4 T× 2 plates	8 T× 2 plates	1 T× 20 plates	16 T× 2 plates	
The magnetic rod sleeve	4 pcs	4 pcs	10 pcs	4 pcs	

Table 3 Main components of BF-T

Components of Kit	32 T/Kit	96 T/Kit	Note
Pre-treatment Solution	3.2 mL× 1 bottle	9.6 mL× 1 bottle	Applicable to semi-automated nucleic acid extraction equipment.
Proteinase K	480 μL× 1 tube	1440 μL× 1 tube	
96-Well Plates Prepackaged Extraction Reagent I	32 T× 1 plate	96 T× 1 plate	
96-Well Plates Prepackaged Extraction Reagent II	32 T× 1 plate	96 T× 1 plate	
96-Well Plates Prepackaged Elution Buffer	32 T× 1 plate	96 T× 1 plate	
96-Well Plates Prepackaged Magnetic Beads Solution	32 T× 1 plate	96 T× 1 plate	

Storage and validity

1. Stored at 2~8°C for 12 months, protecting from direct sunlight and moisture.

2. Once opened, proteinase K should be stored at 2~8°C and the remaining components can be stored at room temperature for 60 days.

Applicable instruments

1. Manual operation: magnetic separator, centrifuge, dry bath.

2. Nucleic acid extraction system: automatic or semi-automatic nucleic acid extraction system based on magnetic beads absorption principle.

Sample requirements

1. Sample types: Cotton swabs, cotton swabs leachate, body fluids, sputum, bacterial cultures, etc.

2. Sample collection: Collect sample via routine method for each sample type.

3. Sample preservation and transportation: The collected samples should be used for nucleic acid extraction immediately, or stored at 2~8 °C (less than 48 hours), at -20 °C for long-term preservation, prevent from freeze-thaw cycles. The samples should be transported in sealed cooler box or styrofoam box with ice seal. The product after extraction should be used immediately for subsequent testing, or stored at 2~8 °C (less than 48 hours), at -20 °C (less than 12 months).

Nucleic Acid Extraction Kit (Magnetic Bead Method)

Pre-treatment

Open the dry bath and set the temperature to 90°C. Place [pre-treatment solution] on the dry bath so that the precipitation dissolves quickly.

1. Cotton swabs

1.1 Take out the 1.5 mL centrifuge tube corresponding to the number of samples and mark it. Add 1 mL cleaning fluid (customers need to prepare, such as physiological saline, TE buffer, pure water, etc.) to the cotton swab sample. After shock for 5 min, pour the liquid into the corresponding marked 1.5 mL centrifuge tube.

1.2 Put the tube into centrifuge, 13000 rpm for 2 min, then discard the 900 μL supernatant. Add 100 μL mixed [Pre-treatment Solution], cover the tube lid, shake and mix well for 10 s, centrifuge it briefly.

1.3 Heat at 90°C for 5 min, centrifuge it briefly.

2. Cotton swabs leachate, bacterial culture, body fluids

2.1 Take out the 1.5 mL centrifuge tube corresponding to the number of samples and mark it. Move 1mL samples into each tube, Put the tube into centrifuge, 13000 rpm for 2 min, then discard the 900 μL supernatant. Add 100 μL mixed [Pre-treatment Solution], cover the tube lid, shake and mix well for 10 s, centrifuge it briefly.

2.2 Heat at 90°C for 5 min, centrifuge it briefly.

3. Sputum (Not liquefied)

3.1 Take out the 1.5 mL centrifuge tube corresponding to the number of samples and mark it. Cut off the head of 1 mL tip, move 200 μL samples into each tube, add 100 μL mixed [Pre-treatment Solution], cover the tube lid, shake and mix well for 10 s, centrifuge it briefly.

3.2 Heat at 90°C for 5 min, centrifuge it briefly.

4. Sputum (Liquefied)

4.1 Take out the 1.5 mL centrifuge tube corresponding to the number of samples and mark it. Move 1 mL samples into each tube. Put the tube into centrifuge, 13000 rpm for 2 min, then discard the 900 μL supernatant. Add 100 μL mixed [Pre-treatment Solution], cover the tube lid, shake and mix well for 10 s, centrifuge it briefly.

4.2 Heat at 90°C for 5 min, centrifuge it briefly.

Test method

1. Manual Operation (Figure 1)

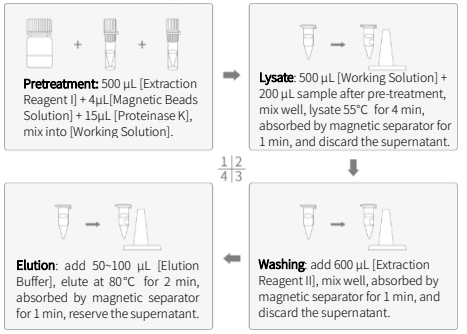


Figure 1 Manual Operation for BF-A

1.1 Take out all the components in the kit, keep them at room temperature and mix them well to be ready for use. If there is a small amount of crystal in [Extraction Reagent I], reagent cannot be used until it is fully dissolved.

1.2 According to the total number of samples, preparation for [Working Solution] : 500 μL [Extraction Reagent I] + 4 μL [Magnetic Beads Solution] + 15 μL [Proteinase K] for each test. Add reagents and solutions proportionally and make them well-mixed. (Note: [Working Solution] should be used within 30 minutes.)

1.3 Add 500 μL [Working Solution] and 200 μL sample after pre-treatment to a marked 1.5 mL centrifuge tube. Shake and mix it up and down for 5 s, then heat it for 4 min on a dry bath at 55°C.

1.4 Centrifuge the tube for 5 s, place it on the magnetic separator for 1 min, then discard the supernatant. (Note: try not to touch the magnetic beads on the tube walls when discarding the supernatant.)

1.5 Add 600 μL [Extraction Reagent II], cover the tube lid, shake and mix well for 5 s. Centrifuge it and place it on the magnetic separator for 1 minute, then discard the supernatant. (Note: the same as step 1.4)

1.6 Remove the residual liquid at the bottom of the tube after 1 min standing.

1.7 Add 50~100 μL [Elution Buffer], cover the tube lid, shake and mix well for 5 s, and centrifuge it briefly.

1.8 Place the centrifuge tube on a dry bath at 80°C, heat for 2 min.

1.9 Place the centrifuge tube on the magnetic separator, and take out supernatant for following operation.

2. Operation of Semi-Automatic Nucleic Acid Extraction System

2.1 For BF-B:

2.1.1 Take out all the components in the kit, mix the 96-well plates upside down so that dump the liquid that adheres to the aluminum film and the well wall of the 96-well plates to the bottom of the plates. Let them stand for 3-5 minutes.

2.1.2 Carefully open the aluminum film of the 96-well plates, and add 15 μL [Proteinase K] to the position A1-H1 and A7-H7 in order, then add 200 μL sample after pre-treatment in order.

Exception: For BF-B-20, carefully open the aluminum film of the 6-well reagent cards, and add 15 μL [Proteinase K] and 200 μL sample after pre-treatment to the position 1 in order. Then put the reagent cards into the baseplate crosswise, put the baseplate into corresponding position of the instrument, and insert the magnetic rod sleeve, the next operations are the same.

2.1.3 Turn on the nucleic acid extraction system, enter the page < Program Edit >, and set the extraction process according to table 4:

Table 4 Running Program Setting

No	Position	Name	Waiting Time (min)	Mixing Time (min)	Absorption Magnetic Beads Time (sec)	Mixture Velocity	Volume	Temperature State	Temperature (°C)
1	2	Move	0	0	30	Slow	150	Closed	0
2	1	Lysis	0	4	60	Slow	500	Heating for Lysis	55
3	3	Wash	0	1	60	Slow	600	Closed	0
4	6	Elution	0	2	30	Slow	50	Heating for elution	80
5	1	Move	0	0	0	Slow	300	Closed	0

(Note: It is recommended to set the parameters of volume according to the actual isolation interaction. The parameters may not be the same with reagent volumes.)

2.1.4 Click "Start" to run the extraction program. The process takes about 10 minutes.

2.1.5 Take out 96-well plates and pipette inventory nucleic acid solution from the position A6-H6 and A12-H12 into 1.5 mL centrifuge tube for following operation. (A small amount magnetic beads could be removed by centrifuge or magnetic separator.)

2.2 For BF-T:

2.2.1 Preparation: Data cable, Computer, Extraction program, Nucleic acid extraction kit (BF-T).

2.2.2 Connect instrument and computer, then import the extraction program: Home- Connet- Transfer...- Upload- Chose the program- Change the name "zhong.yuan" to "zhongyuan" (remove " . "), import into folder.

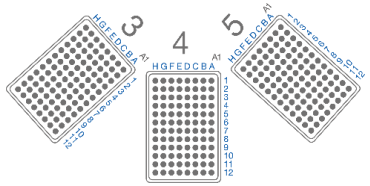
2.2.3 Adding sample

Add 15 μL [proteinase K] and 200 μL sample to [Extraction reagent I].

2.2.4 Instrument operation

Chose the extraction program "zhongyuan" - Click "Start" - Loading reagents:

1) Put [Elution buffer] plate to position 4;
2) Put [Extraction Reagent II] plate to position 3;
3) Put [Extraction Reagent I] plate to position 2;
4) Put [Magnetic Bead Solution] plate to position 1, and then put the magnetic rod sleeve into the [Magnetic Bead Solution] plate.



Note: The placement of the 96-well plates are as shown below. After the end of running, take out the corresponding 96-well plate ([Elution buffer] plate) for subsequent experiment according to the instrument prompt.

3. Operation of Automatic Nucleic Acid Extraction System

3.1 Take out all the components in the kit, keep them at room temperature and mix them well to be ready for use. If there is a small amount of crystal in [Extraction Reagent I], reagent cannot be used until it is fully dissolved.

3.2 Refer to the operation manual and Standard of Operation of the automatic nucleic acid extraction system to complete the extraction of nucleic acid.

Limitations on test method

This product needs to be used with a magnetic separator in manual operation.

Product performance index

1. Appearance: The outer packing is printed accurately and intact. There are complete components in the kit, no obvious impurities, the packaging appearance is clean, no leakage, no damage. Labels and insert are complete and accurate. [Pre-treatment Solution], [Extraction Reagent I], [Extraction Reagent II], and [Elution buffer] were colorless and transparent solutions. [Pre-treatment Solution] and [Extraction Reagent I] may have precipitate at low temperature. [Magnetic Beads Solution] was black brown particle suspension, and [Proteinase K] was light yellow transparent solution.

2. Yield: Take 8 cases of 1 mL e.coli (OD_{600} = 1), the total amount of DNA isolated from each sample is ⩾ 2 μg.

3. Extracted analyte purity: $OD_{260/280}$ of the extracted analyte should be between 1.6 and 2.0.

Warnings and precautions

1. The components of the kit needs to be mixed well before use. If the [Pre-treatment Solution] is cloudy, it can be used normally after shaking it well, or using a dry bath to make it melt to clarify. If there is a small amount of crystal in [Extraction Reagent I], reagent cannot be used until it is fully dissolved.
2. Considering [Washing Solution] contains flammable components, please keep away from fire sources or other risk factors.
3. Clinical samples may have biological hazards. Pre-treatment process suggests operation in the biosafety cabinet.
4. The disposal of waste liquid should be in accordance with local laws and regulations.

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. Sonja B. Magnetic particles for the separation and purification of nucleic acids. Appl Microbiol Biotechnol, 2006, 73:495-504.
3. Li J M. Real-time Fluorescent PCR Technology [M]. Beijing: People's Military Medical Press, 2007.

Explanations on symbols

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		Date of manufacture

zybio

Nucleic Acid Extraction Kit (Magnetic Bead Method)

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' SREPUBLIC 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 Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany