IVD CE

bosphore®

Nucleic Acid

Extraction Versatile Spin Kit

User Manual

For *in vitro* Diagnostic Use

MB325v5f
May 2019

Anatolia geneworks

CONTENTS

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1. PRODUCT DESCRIPTION

Bosphore Nucleic Acid Extraction Versatile Spin Kit has been designed for manual extraction of nucleic acids from various samples. The kit contains the required solutions for extraction as well as the plastic consumables for spin column. Bosphore Nucleic Acid Extraction Versatile Spin Kit is compatible with Bosphore® Real-Time PCR kits.

2. CONTENT

Bosphore® Nucleic Acid Extraction Versatile Spin Kit is composed of the following components.

Component	Content	Amount (100 Extractions)
1	Buffer LB1	40 ml
2	Buffer IR2	100 ml
3	Buffer W3	100 ml
4	Buffer EL4	10 ml
5	Proteinase K	11 mg x 4
6	PK Storage Buffer	1.25 ml x 4
7	Spin columns	100 pieces
8	Collection tubes	400 pieces
9	Carrier RNA	1 mg

3. STORAGE

All of the Bosphore Nucleic Acid Extraction Versatile Spin Kit components are stored at room temperature. After suspension, Proteinase K should be stored at +4°C.

Kit components maintain their stability until the expiry dates on their labels, if they are stored at recommended conditions.

4. REQUIRED MATERIALS AND DEVICES

- Ethanol (96–100%)
- RNase A *
- Carrier RNA
- Buffer LTX *
- Refrigerator (+4°C)
- Thermomixer or thermalblock
- Vortex
- Desktop centrifuge with rotor for 2 ml. microcentrifuge tubes
- Calibrated adjustable micropipettes
- DNAse, RNAse, pyrogen free micropipette tips with filters
- DNAse, RNAse, pyrogen free 1.5 or 2 ml. microcentrifuge tubes
- Disposable laboratory gloves

*** Contact Anatolia Geneworks for the required reagents.**

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

Important! :

- Check for the expiry dates on the box and tube labels, upon arrival. Do not use expired products or components.

- Calibrated or verified micropipettes, DNAse, RNAse, pyrogen free micropipette tips with filters, and DNAse, RNAse; pyrogen free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be mixed well to ensure homogeneity prior to use.
- PCR and nucleic acid isolation must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Biological samples should be handled with extreme caution: Physical contact with pathogens should be avoided by: wearing lab coats and gloves, no allowance for eating or drinking within the workspace, prevention of unauthorized individuals' access to the working area.
- Pathogen information should be reviewed to be aware of the health related risks.
- All the pathogenic wastes produced during the nucleic acid isolation step; including the biological samples and material contacted with them, should be discarded into the medical waste and disposed safely.

6. PRODUCT USE LIMITATIONS

- This product may exclusively be used for in vitro diagnostics.
- This product should be used by personnel specially trained to perform in vitro diagnostic procedures accordance with this user manual.

7. METHOD

Bosphore Nucleic Acid Extraction Versatile Spin Kit is based on the silica membrane column separation method. The kit involves extraction of nucleic acids from biological samples of patients by removing and purifying the nucleic acids from cell components. In this manner, DNA isolation steps include disruption/lysis of the samples, inactivation of nuclease activity, binding of the DNA to the silica membrane, removal of the contaminants and recovery of the nucleic acid respectively. So far, methods generally employed have been considered in two categories; the conventional 'liquid-phase isolation' and common 'solid-phase isolation' which is highly appreciated in molecular biology field as a result of its practical use. Liquid-phase isolation methods include; Guanidinium Acid - Phenol-Extraction method, Alkaline Extraction method, Ethidium Bromide (EtBr)-Cesium Chloride (CsCl) Gradient centrifugation method and Oligo(dT)-Cellulose Chromatography method. Recently, Solid-phase isolation methods are preferred as they avoid the phase separation problems usually faced in 'liquid-phase isolation' methods,

and are faster and more effective than conventional methods.

Isolation of the nucleic acid is carried out in 4 main steps that involve lysis, binding, wash and elution. During the lysis/disruption step, the samples are lysed by using a lysis buffer solution with excessive denaturing conditions that inactivate the DNAse and RNAse activity. Using a buffer at a particular pH, the surface or functional groups on the solid/column are converted into a particular chemical form. Buffer conditions (pH and salt concentration) are optimised in order to achive the effective binding of nucleic acid to the column surface. Other components are removed in further washing steps using the wash buffer. Mostly, alcohol solutions are applied during these wash steps. The components apart from nucleic acids are easily removed from nucleic acids as they tend to dissolve in alcohols. After this step, extraction is completed by eluting the bound DNA using elution buffer. As Elution buffer, water or TE buffer is mainly used and nucleic acids are recovered during this last step.

The ion concentrations and pH degrees of the solutions used in nucleic acid extraction play an important role. The used solid-surface material is very important. The main solid-surface (suport) materials used in solid-phase extraction systems are usually; silica matrix, glass particles, diatomite, magnetic particles or anion-change surfaces. Nowadays, most of the commercial manual extraction kits available in the market are based on silica based column

technology. In this technology, nucleic acids tend to bind to the silica column and separated from other components by applying centrifuge.

8. PROCEDURE

Before use;

- 1.1ml PK Storage Buffer is added to the Proteinase K tube and mix by vortexing.

Proteinase K (10mg/ml) solution is ready to use and should be stored at +4°C.

Caution!:

If you observe precipitation in Buffer LB1 and Buffer IR2, Please heat the solution and dissolve the precipitate.

Extraction protocol should be performed by carefully applying the solutions to the center of the membrane, and the tip of the micropipette should not touch the spin column membrane.

8.1. Nucleic Acid Extraction From Whole Blood

1. Mix 20 µl of Proteinase K, 250 µl of Buffer LB1 and 250 µl of Whole Blood sample in a 1.5 ml microcentrifuge tube and mix by vortexing for 15 s.

2. Incubate for 15 min. at room temperature. **(<u>Lysis Step</u>)**

3. Add 250 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s.

4. Apply all of the mixture to the spin column and centrifuge at 11.000 rpm for 1 min.
(<u>Binding Step</u>)

5. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube. **(If there is some liquid left on top of the spin column after the centrifugation, place the spin column in a new collection tube and centrifuge at 11.000 rpm for 1 min for a second time.)**

6. Add 500 µl of Buffer IR2 to the spin column and centrifuge at 10.000 rpm for 1 min.
(Wash Step #1)

7. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

8. Add 500 µl of Buffer W3 to the spin column and centrifuge at 10.000 rpm for 1 min.
(Wash Step #2)

9. Discard the liquid flow-through into medical waste and place the spin column back into the same collection tube

10. Add 500 µl of Buffer W3 to the spin column at 10.000 rpm for 1 min. **(Wash Step #3)**

11. Discard the liquid flow-through into medical waste and place the spin column back into the same collection tube

12. Centrifuge the empty spin column at 12.500 rpm for 2 min.

13. Discard the collection tube including the liquid flow-through under the spin column into medical waste

14. Place the spin column in a clean 1.5 ml microcentrifuge tube, open the cap of the spin column and wait for 1 min. to evaporate any remaining EtOH.

15. Add 60-100 µl Buffer EL4 to the spin column and wait for 5 min at room temparature. **(<u>Elution Step</u>)**

16. Centrifuge at 12.500 rpm for 1 min.

17. The liquid flow-through is the nucleic acid sample. Store the nucleic acid at +4°C in case of instant use or store at -20°C for later use.

8.2. Nucleic Acid Extraction From Tissue Samples

1. Cut 100 – 250 mg of fresh or frozen tissue sample. Grind the tissue and transfer it to a 1.5 ml microcentrifuge tube

2. For each sample, add 450 µl of Buffer LTX **(not supplied)** and 20 µl of Proteinase K and mix by vortexing.

3. Incubate the mixture for at least 60 min. at 56°C **(Lysis Step)**

4. Centrifuge the mixture at 12.500 rpm for 5 mins.

5. **Transfer 400** µl of **the clear supernatant to a new** microcentrifuge tube.

- **Optional RNase A Digestion: an optional RNase A digestion step to remove the RNA can be made at this step, if the RNA in the eluate interferes with the related downstream application. Otherwise continue the extraction with step 6. For this purpose add 2.6 µl of 50 mg/ml RNase A (not supplied within the kit!) to the**

clear supernatant, mix and incubate at room temp for at least 10 mins.

6. Add 400 µl of Buffer LB1 and 400 µl of ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s.

7. Apply 650 µl of the mixture to the spin column and centrifuge at 10.000 rpm for 1 min.
(Binding Step)

8. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

9. Apply the remaining of the mixture to the spin column and centrifuge at 10.000 rpm for 1 min.
(Binding Step)

10. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

11. Add 500 µl of Buffer IR2 to the spin column and centrifuge at 10.000 rpm for 1 min.
(Wash Step #1)

12. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

13. Add 500 µl of Buffer W3 to the spin column and centrifuge at 10.000 rpm for 1 min. **(Wash Step #2)**

14. Discard the liquid flow-through into medical waste and place the spin column back into the same collection tube

15. Add 500 µl of Buffer W3 to the spin column and centrifuge at 10.000 rpm for 1 min. **(Wash Step #3)**

16. Discard the liquid flow-through into medical waste and place the spin column back into the same collection tube

17. Centrifuge the empty spin column at 12.500 rpm for 2 min.

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18. Discard the collection tube including the liquid flow-through under the spin column into medical waste

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19. Place the spin column in a clean 1.5 ml microcentrifuge tube, open the cap of the spin column and wait for 1 min. to evaporate any remaining EtOH.

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20. Add 60-100 µl Buffer EL4 to the spin column and wait for 5 min at room temparature.

(<u>Elution Step</u>)

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21. Centrifuge at 12.500 rpm for 1 min.

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22. The liquid flow-through is the nucleic acid sample. Store the nucleic acid at +4°C in case of instant use or store at -20°C for later use.

8.3. Viral RNA & DNA Extraction

- We strongly recommend using serum samples for viral nucleic acid extraction in order to provide higher sensivity and reproducibility.
- Resuspend 1 mg of Carrier RNA with 1 ml of DNase/RNase free water provided

1. Prepare an extraction mix with 40 µl of Proteinase K, 10 µl of Carrier RNA and the related internal control for each sample and divide the extraction mix into the one set of labeled microcentrifuge tubes.

2. Add 400 µl Serum/Plasma sample onto the Extraction Mix and mix by pipetting.

3. Add 400 µl of Buffer LB1 on each sample and mix by vortexing. Incubate the mixture for 10 min. at 56˚C

(Lysis Step)

4. Add 500 µl of Buffer IR2 to the mixture, mix by pipeting and incubate for 5 min. at room temperature.

(Inhibitor removal)

5. Apply 650 µl of the mixture to the spin column and centrifuge at 8.000 rpm for 1 min.

(Binding Step)

6. Discard the liquid flow-through into medical waste and place the spin column in a new collection tube.

7. Apply the remaining of the mixture to the spin column and centrifuge at 8.000 rpm for 1 min.

(Binding Step)

8. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

9. Add 500 µl of Buffer W3 to the spin column

(Wash Step #1)

10. Centrifuge at 8.000 rpm for 1 min.

11. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

12. Add 500 µl of Buffer W3 is added to the spin column **(<u>Wash Step #2</u>)**

13. Centrifuge at 8.000 rpm for 1 min.

14. Discard the liquid flow-through together with the collection tube and spin column back into the same collection tube

15. Centrifuge the empty spin column at 12.500 rpm for 2 min.

16. Discard the collection tube including the liquid flow-through under the spin column into medical waste

17. Place the spin column in a clean 1.5 ml microcentrifuge tube, open the cap of the spin column and wait for 1 min. to evaporate any remaining EtOH.

18. Add 60-100 µl of Buffer EL4 to the spin column and wait for 5 min. **(<u>Elution Step</u>)**

19. Centrifuge at 12.500 rpm for 1 min

20. The liquid flow-through is the DNA & RNA sample. Store the sample at +4°C in case of instant use or store at -20°C for later use.

8.4. Bacterial RNA & DNA Extraction

8.4.1. Sample preparation:

Various swab samples in liquid transport media: prior to extraction homogenize the sample thoroughly by shaking. Take 400 µl of the liquid and follow the extraction protocol below.

Dry swabs: Insert the tip of the cotton swab into a sterile microcentrifuge tube containing 1000 - 500 µl of PBS or just water. The volume should be enough to allow total immersion of the cotton. Incubate for 5 minutes with pressing the swab is against the edges of the tube often. This will transfer the sample in to the liquid and then the swab is removed. Take 400 µl of the liquid and follow the extraction protocol below.

Bodyfluids (such as serum, urine, whole blood etc.) prior to extraction homogenize the sample thoroughly. Take 400 µl of the bodyfluid and follow the extraction protocol below. If needed, centrifuge the sample for up to 10 min at 12500 rpm to concentrate the bacterial cells in pellet. Discard supernatant, resuspend the pellet in 400 µL PBS or dh2o and follow the extraction protocol below.

Viscous samples (BAL, sputum or other mucous specimen). It is recommended to perform decontamination before DNA extraction. Commercial decontamination products and kits are compatible with Bosphore Nucleic Acid Extraction Versatile Spin Kit. If in-house methods are preferred for the decontamination step, decontamination could be done by using solutions, such as 4% NaOH and 1% N-Acetylcystein.

Resuspend the bacterial pellt at the end of the decontamination procedure with 400 µL PBS or dh2o and follow the extraction protocol below.

Solid samples (various tissue or stool specimens):

Cut 100 – 250 mg of fresh or frozen specimen. Grind the specimen and transfer it to a 1.5 ml microcentrifuge tube

For each sample, add 450 µl of Buffer LTX **(not supplied) and** 20 µl of Proteinase K and mix by vortexing.

Incubate the mixture for at least 60 min. at 56◦C (Lysis Step)

Centrifuge the mixture at 12.500 rpm for 5 mins.

Transfer 400 µl of the clear supernatant to a new microcentrifuge and follow the extraction protocol below (Use 20 µl of Proteinase K instead of 40 µl at the first step).

1. Mix 40 µl of Proteinase K, 400 µl of Buffer LB1 and 400 µl of sample in a 1.5 ml microcentrifuge tube and mix by vortexing

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2. Incubate for 15 min. at 56°C **(Lysis Step)**

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3. Add 500 µl of Buffer IR2 to the mixture, mix by pipeting and incubate for 5 min. at room temperature. **(Inhibitor removal)**

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4. Apply 650 µl of the mixture to the spin column and centrifuge at 10.000 rpm for 1 min.

(Binding Step)

5. Discard the liquid flow-through into medical waste and place the spin column in a new collection tube.

6. Apply the remaining of the mixture to the spin column and centrifuge at 10.000 rpm for 1 min.

(Binding Step)

7. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

8. Add 500 µl of Buffer IR2 to the spin column and centrifuge at 10.000 rpm for 1 min.

(Wash Step #1)

9. Discard the liquid flow-through together with the collection tube and place the spin column in a new collection tube.

10. Add 500 µl of Buffer W3 to the spin column
(<u>Wash Step #1</u>)

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11. Centrifuge at 10.000 rpm for 1 min.

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12. Discard the liquid flow-through together with the collection tube and place spin column back into the same collection tube

⇩

13. Add 500 µl of Buffer W3 is added to the spin column
(<u>Wash Step #2</u>)

⇩

14. Centrifuge at 10.000 rpm for 1 min.

⇩

15. Discard the liquid flow-through together with the collection tube and place spin column back into the same collection tube

⇩

16. Centrifuge the empty spin column at 12.500 rpm for 2 min.

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17. Discard the collection tube including the liquid flow-through under the spin column into medical waste

18. Place the spin column in a clean 1.5 ml microcentrifuge tube and wait for 1 min to evaporate EtOH.

19. Add 100 – 150 µl of Buffer EL4 (*) to the spin column and wait for 5 min. **(<u>Elution Step</u>)**

20. Centrifuge at 12.500 rpm for 1 min

21. The liquid flow-through is the DNA & RNA sample. Store the sample at +4°C in case of instant use or store at -20°C for later use.

** It is recommended to use 150 µl Buffer EL4 for extraction from samples of Mycobacterium tuberculosis complex*

9. SYMBOLS

 Use-by date

 Batch code

 Catalogue number

 Caution

 Manufacturer

 *In Vitro* Diagnostic Medical Device

10. ORDERING INFORMATION

Catalog Number: **ABXGV1 (100 extractions/box)**

11. CONTACT INFORMATION

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. Ve Tic. A.S.

Address: Eğitim Mah. Kasap İsmail Sk
Istanbul Plaza
No:10/23 Kadikoy 34722
ISTANBUL-TURKEY
Phone: +90 216 330 04 55
Fax: +90 216 330 00 42
E-mail: info@anatoliageneworks.com
www.anatoliageneworks.com