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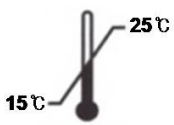
ExiPrep™ Bacteria Genomic DNA Kit

User's Guide

REF K-3245



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ExiPrep™ Bacteria Genomic DNA Kit

Kit for the extraction of bacteria genomic DNA in various samples using *ExiPrep*™ instrument

User's Manual

Σ 96

Version No.: 3.0 (2010-12-24)

BIONEER CORPORATION
49-3 Munpyeong-Dong, Daedeok-Gu, Daejeon, 306-220, Korea
Tel: +82-42-930-8777
Fax: +82-42-930-8600
Email: order@bioneer.co.kr
www.bioneer.com

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Safety Warnings and Precautions

Please inquire BIONEER's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

Before, during and after use of this kit as described in this User Manual, all potentially hazardous materials (i.e. materials that may have come in contact with clinical samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/government in which this product is being used.

Please read the User Manual before using this Kit. Please check the integrity of all tubes, tips and other materials supplied with this kit prior to use. Adhere to general clinical laboratory safety procedures during the experiment.

Warranty and Liability

All BIONEER products are manufactured and tested under strict quality control protocols certified under **ISO 9001** and **ISO 13485** standards. BIONEER guarantees the quality of all directly manufactured products during the warranty period of one (1) year from date of purchase. If any issues are discovered relating to compromise in product quality, immediately contact BIONEER's Customer Service Center (order@bioneer.com).

Trademarks

ExiPrep™ is the trademark of Bioneer Corporation. KOREA

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1. Kit components

	ExiPrep™ Bacteria Genomic DNA Kit (K-3245)
Buffer Cartridge ①	6 ea
Buffer Cartridge ②	6 ea
Resuspension Buffer	1 ea
Disposable Tip	3 pack
Elution Tube (8-strip)	1 pack
User's Manual	1 ea
One Page Protocol	1 ea

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2. Storage

ExiPrep™ Kits are designed to contain all necessary buffers (including binding/washing/elution buffers) and components within the Buffer Cartridges. All Buffer Cartridges are sealed with protective film to prevent leakage, evaporation and contamination. The Buffer Cartridges can be stored at room temperature (15℃-25℃) under dry conditions for up to 2 years without opening.

ExiPrep™ Kits may also provide enzymes in lyophilized form for increased convenience.

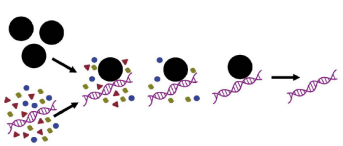
Lyophilized enzymes may be contained within the Buffer Cartridges and/or 2.0mL screw cap tubes. The enzymes in lyophilized form can be stored at room temperature (15℃-25℃) up to 2 years without any detectable loss in activity. Reconstituted enzymes must be stored at -20℃

The disposable tips and elution tubes included within the Kit are DNase and RNase free. Please take caution in storing the consumables and avoid nuclease contamination.

3. Introduction

ExiPrep™ Bacteria Genomic DNA Kit is designed for the extraction of genomic DNA from gram negative and positive bacteria and fungi. Gram positive bacteria and fungi must undergo an enzymatic digestion step for optimal extraction. The digested samples them must be resuspended with the provided Resuspension Buffer before proceeding with genomic DNA extraction.

Samples are mixed with Lysis Buffer contained within Buffer Cartridge ① and undergo incubation so the nucleic acids may be released into the solution. After the incubation step, silica magnetic beads will be introduced to bind the DNA. The magnetic beads can be separated from the solution (containing cellular debris) through magnetization. The beads are then washed to flush any impurities that may exist. Finally, a small volume of elution buffer washed over the magnetic beads will release the DNA. The eluted DNA is in a highly pure form and is ready to be used.



[Description of DNA extraction using silica magnetic bead]

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4. Starting Sample & Typical yield

Typical amounts of starting material quantity, elution volume and the typical yields are described below. Results of your application may vary.

Sample type	Starting Sample	Elution Volume	Typical Yield
Gram (-) bacteria	~1x10^{8}cells	50~200ul	5-15ug
Gram (+) bacteria	~1x10^{8}cells	50~200ul	5-15ug
Yeast (*S. pombe*)	~1x10^{8}cells	50~200ul	5-15ug
Fungi	~1x10^{8}cells	50~200ul	5-15ug

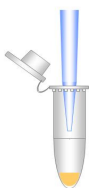
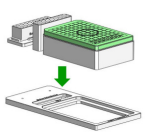
5. Genomic DNA Extraction

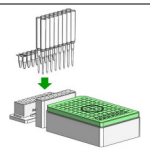
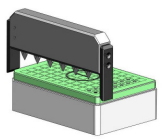
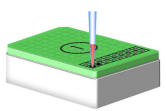
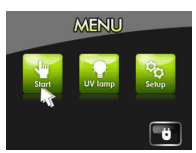
5.1 Additionally required materials & devices

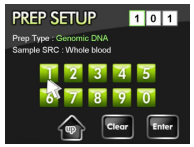
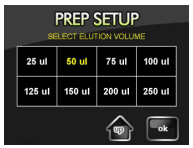
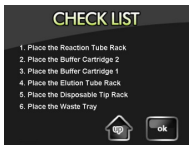
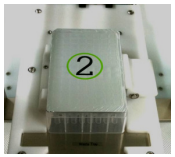
- Disposable gloves
- Pipettes
- Sterilized pipette tips with filters
- 1.5ml microcentrifuge tubes or 15ml conical tubes
- 1x PBS buffer, 1x TE buffer, 10N NaOH
- Lysozyme or lyticase
- Table top centrifuge or swing rotor centrifuge
- *Exiprep*™ 16 Plus (Cat. No. : A-5030, Bioneer Co.,KOREA), *Exiprep*™ 16 Pro (Cat. No. : A-5040, Bioneer Co.,KOREA)

5.2 Extraction from cultured bacteria

This protocol is designed for extraction of genomic DNA from cultured bacteria.

	1. **a. For Gram (-) bacteria** Resuspend the cell pellet (up to 1x10^8 cells) in 200ul of Resuspension Buffer. **b. For Gram (+) bacteria** - Resuspend the cell pellet (up to 1x10^8 cells) in 200ul of 1x TE buffer - Add 20ul of lysozyme (50mg/ml) and incubate the tube at 37℃ for at least 1hr. - Centrifuge the tube at 5000rpm for 3min and remove the supernatant. - Re-suspend the pellet with 200ul of Resuspension Buffer.
	2. Place the elution tube rack, tip rack, and Buffer Cartridge ① on the setup tray.

	3. Ensure that all tips and tubes are inserted in the desired positions.
	4. Punch holes through the Buffer Cartridge sealing film with the 6-hole punch tool in the locations corresponding to the tubes and tips. ※ Before punching the holes, make sure to shake Buffer Cartridge ① to suspend the beads, then gently swing outward to settle solutions to the bottom of the wells.
	5. Load 200 µL of sample into the sample loading wells.
	6. Turn on the *ExiPrep*™ instrument 7. Press the **'Store'** button for cooling. (*ExiPrep*™ 16 Pro only) 8. Press the **'Start'** button to access the **PREP SETUP** menu.

	9. Enter a protocol number from the protocol list (Chapter 7) that best suits your sample source and target nucleic acid type. 10. Press the **'Enter'** button to proceed to the next step.
	11. Select the desired elution volume from the screen. ※ Recommended elution volume: 100ul 12. Press the **'ok'** button to move to the next step.
	13. Open the instrument door and pull out the Base Plate. 14. Place all racks and Buffer Cartridges in their correct positions on the Base Plate according to the **CHECK LIST** (steps 15~19).
	15. Place Buffer Cartridge ② into its labeled position on the Base Plate.

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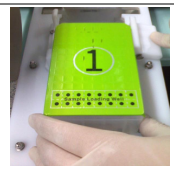
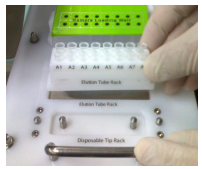
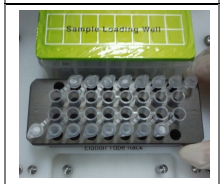
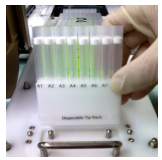
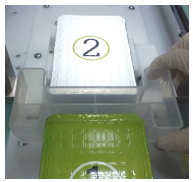
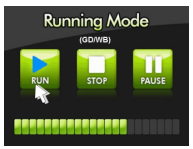
	16. Place Buffer Cartridge ① into its labeled position on the Base Plate.
 	17. Place the Elution Tube Rack into its labeled position on the Base Plate. **a. For *ExiPrep*™ 16 Plus** Place the Elution Tubes in Lanes A and B of the Elution Tube Rack. **b. For *ExiPrep*™ 16 Pro** Place the Elution Tubes into the 2nd and 3rd rows of the Elution Tube Rack.
	18. Place the Disposable Filter Tip Rack into its labeled position on the Base Plate.
	19. Place the Waste Tray into its proper position (between Buffer Cartridges ① and ②). 20. Push the Base Plate completely back into the instrument and close the door.

	21. Verify the protocol name on the screen. The first two letters represent the target nucleic acid type, and the next two letters represent the sample source. 22. Press the **'Run'** button to start the extraction run.
	23. When the run process is successfully completed, three options will display.
	24. Open the door and pull out the Base Plate. 25. Take the Elution Tubes from Base Plate. 26. Remove the Buffer Cartridges and all racks from Base Plate and close the door. 27. Quantify the purified genomic DNA (if desired).

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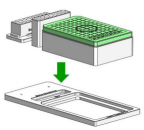
5.3 Extraction from urine

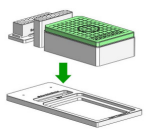
This protocol is designed for the extraction of genomic DNA from urine samples.

	1. Transfer an adequate volume of urine into a 15ml conical tube. 2. Centrifuge at 3,000 rpm for 20 min. ※ Please repeat centrifugation if pelleting is insufficient. 3. Add 200µl of Resuspension Buffer and mix well by vortexing for 15 sec. ※If the sample has excessive sedimentation, the volume of Resuspension Buffer may be increased up to 500µl.
	4. Go to step 2. of '5.2 Extraction from cultured bacteria'. ※ Tip: These steps may be prepared during the sample centrifugation step.

5.4 Extraction from sputum

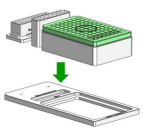
This protocol is designed for the extraction of genomic DNA from sputum samples.

	1. Add 1/10 volume of 10N NaOH into the container holding the sputum sample and mix by vortexing for 3 min. Incubate the sample tubes for 15 min at room temperature. ※ If sample has high viscosity, add more 10N NaOH (up to 500µl) or incubate the sample tube for up to 30 min.
	2. Transfer 1.5ml of sample into a 1.5ml microcentrifuge tube. 3. Centrifuge the tube at 9,000 rpm for 5 min and completely remove the supernatant. ※ If your centrifugation is incomplete, please repeat centrifugation.

	4. Re-suspend the pellet with 1ml of 1X PBS buffer. 5. Centrifuge the tube at 9,000 rpm for 5 min and completely remove the supernatant. ※ 6. Repeat step 4.~5. over two times. 7. Resuspend the pellet with 200µl of Resuspension Buffer.
	8. Go to step 2. of '5.2 Extraction from cultured bacteria'.

5.5 Extraction from swab

This protocol is designed for the extraction of genomic DNA from swab samples.

	1. Transfer the swab into a 15ml conical tube or a specialized sample tube. 2. Add 1ml of 1x PBS buffer or nomal saline and mix well by vortexing. ※ If the specialized sample tube contains media, do not add the 1ml liquid and proceed to the next step.
	3. Transfer 500µl of sample into a 1.5ml microcentrifuge tube. 4. Centrifuge the tube at 13,000 rpm for 2 min and remove the supernatant. 5. Resuspend the pellet with 200µl of Resuspension Buffer.
	6. Go to step 2. of '5.2 Extraction from cultured bacteria'.

6. Troubleshooting

A. Low yield of Genomic DNA

1) Did you add sufficient amount of sample? The yield is dependent on the sample type and amount. Overloading sample may sometimes decrease the yield.
2) Did you lyse the samples completely? Incomplete lysis decreases yield and purity.
3) Is there salt precipitate in any of the buffers? Warm the bottles at 60℃ to re-dissolve.
4) Did you shake Buffer cartridge ① before use? Incomplete resuspension of the magnetic beads may decrease the yield and purity.

B. Co-eluted magnetic particles

The magnetic particles may co-elute with the extracted DNA after genomic DNA extraction. Co-eluted magnetic particles cannot bind nucleic acid in Elution Buffer and it will not decrease the yield and purity.

Co-eluted magnetic particles can easily be separated by simple centrifugation.

C. Empty elution tubes

1) Too much starting sample

   Please add the volume of sample suggested in this manual. Excessive sample volume may cause clogging by magnetic beads and interfere with normal extraction.

2) Insufficient elimination of the supernatant

   When processing samples to prepare them for extraction, you must ensure complete removal of sample supernatant (ex. Lipid layer, medium). Insufficient removal of impurities may cause clogging by magnetic beads and interfere with normal extraction.

D. Expansion of sealing film

Please store the Buffer Cartridges in recommended storage conditions (Chapter 2. Storage). High storage temperatures will increase the vapor pressure of certain buffers and force the film to bulge.

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7. Protocol number list

No.	Target	Sample source
101	Genomic DNA	Whole blood
102	Genomic DNA	Animal tissue
103	Genomic DNA	FFPE tissue
104	Genomic DNA	Plant tissue
105	Genomic DNA	Plant seed
106	Genomic DNA	Rice
107	Genomic DNA	Cultured cell
108	Genomic DNA	Gram (+) bacteria
109	Genomic DNA	Gram (-) bacteria
110	Genomic DNA	Yeast
111	Genomic DNA	Fungi
114	Genomic DNA	Buffy coat
115	Genomic DNA	Sputum
116	Genomic DNA	BAL
117	Genomic DNA	Saliva
118	Genomic DNA	Swab
119	Genomic DNA	Urine
120	Genomic DNA	Stool
123	Genomic DNA	CSF
124	Genomic DNA	EPS
125	Genomic DNA	Respiratory sample

8. Ordering information

Product	Size	Cat. No.
ExiPrep™ 16Plus	1 ea	A-5030
ExiPrep™ 16Pro	1 ea	A-5040
ExiPrep™ Blood Genomic DNA Kit	96 prep.	K-3215
ExiPrep™ Tissue Genomic DNA Kit	96 prep.	K-3225
ExiPrep™ Bacteria Genomic DNA Kit	96 prep.	K-3245
ExiPrep™ Plant Genomic DNA Kit	96 prep.	K-3255
ExiPrep™ Viral DNA Kit	96 prep.	K-3515
ExiPrep™ Viral RNA Kit	96 prep.	K-3525
ExiPrep™ Beef Genomic DNA Kit	96 prep.	K-3200-CB
ExiPrep™ Rice Genomic DNA Kit	96 prep.	K-3200-CR

9. References

Obata, K., Segawa, O., Yakabe, M., Ishida, Y., Kuroita, T., Ikeda, K., Kawakami, B., Kawamura, Y., Yohda, M., Matsunaga, T. and Tajima, H. (2001). Development of a novel method for operating magnetic particles magtration technology, and its use for automating nucleic acid purification. *J. Biosci. Bioeng.*, 91(5), 500–503.

Honore-Bouakline, S., Vincensini, J. P., Giacuzzo, V., Lagrange, P. H., Herrmann, J. L. (2003). Rapid Diagnosis of Extrapulmonary Tuberculosis by PCR: Impact of Sample Preparation and DNA Extraction. *J. Clin. Microbiol.* 41: 2323-2329

Boom, R., Sol, C., Beld, M., Weel, J., Goudsmit, J. and Wertheim-Van Dillen, P. (1999) Improved silica-guanidiniumthiocyanate DNA isolation procedure based on selective binding of bovine alphacasein to silica particles. *Journal of Clinical Microbiology* 37, 615–619

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10. Explanation of symbols

Catalog Number

Batch code

Contains Sufficient for test


Expiration Date


Storage Conditions (Temp.)


Manufacturer


Caution, Consult accompanying documents

• Bioneer World Wide

Headquarters

Address 49-3, Munpyeong-dong, Daedeock-gu, daejeon 306-220, Korea
Tel +82-42-930-8777 (Korea only: 1588-9788)
Fax +82-42-930-8600
E-mail sales@bioneer.co.kr
Web http://www.bioneer.co.kr

Seoul Office

Address 2F Samhwawa Sansu Building, 6, Yangjae-Dong, Seocho-Gu, Seoul 137-130, Korea
Tel +82-2-598-1094 (korea only: 1588-9788)
Fax +82-8-598-1096
E-mail sales@bioneer.co.kr
Web http://www.bioneer.co.kr

Bioneer,Inc.

Address 1000 Atlantic Avenue Alameda, CA 94501 USA
Tel +1-877-264-4300(Toll Free)
Fax +1-510-865-0350
E-mail order.usa@bioneer.com
Web www.bioneer.com

Bioneer Trade (Shanghai) Co, Ltd

Address 403Room, Building 88, no.887, Zuchongzhi Road, Zhangjiang High Tech Park, PuDong New District, Shanghai 201203, China
Tel +86-21-5080-0969
Fax +86-21-5080-1620
E-mail asan@bioneer.com
Web www.bioneer.com