User's Guide

ExiPrep™ Plus Genomic DNA Kit

REF Cat. No.: K-4211 - K-4215

BIONEER
Innovation • Value • Discovery

ExiPrep™ Plus Blood Genomic DNA Kit (K-4211)

ExiPrep™ Plus Tissue Genomic DNA Kit (K-4212)

ExiPrep™ Plus Cell Genomic DNA Kit (K-4213)

ExiPrep™ Plus Bacteria Genomic DNA Kit (K-4214)

ExiPrep™ Plus Plant Genomic DNA Kit (K-4215)

User's Guide

Version No.: 1.0 (2012-11)

Please read all the information in booklet before using the unit

BIONEER CORPORATION
8-11 Munpyeongseo-ro, Daedeok-Gu, Daejeon,
306-220, Korea
Tel: +82-1588-9788
Fax: +82-42-930-8600
Email: order@bioneer.co.kr
www.bioneer.co.kr

Safety Warnings and Precautions

ExiPrep™ Plus Tissue Genomic DNA Kit is developed and sold for research use only. It is not recommended for human or animal diagnostic use unless cleared for such purposes by the appropriate regulatory authorities in the country of use.

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information please consult the appropriate Material Safety Data Sheets (MSDS).

Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Trademarks

ExiPrep™ is a trademark of Bioneer Corporation.

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Contents

I. Kit components

	ExiPrep™ Plus Genomic DNA Kit				
Cat. No	Blood (K-4211)	Tissue (K-4212)	Cell (K-4213)	Bacteria (K-4214)	Plant (K-4215)
Buffer Cartridge ①	6 ea	6 ea	6 ea	6 ea	6 ea
Buffer Cartridge ②	6 ea	6 ea	6 ea	6 ea	6 ea
Tissue Lysis Buffer	-	1 ea	-	-	-
Resuspension Buffer	-	-	1 ea	1 ea	-
Plant Lysis Buffer	-	-	-	-	1 ea
Proteinase K (20.0 mg)	-	2 ea	-	-	2 ea
Disposable Filter Tip	96 ea	96 ea	96 ea	96 ea	96 ea
Elution Tube (8-strip)	12 ea	12 ea	12 ea	12 ea	12 ea
User's Guide	1 ea	1 ea	1 ea	1 ea	1 ea

II. Introduction

The ***ExiPrep***™ **Plus Blood Genomic DNA Kit** is suitable for the extraction of genomic DNA from whole blood, buffy coat, urine or any liquid phase sample using the automatic nucleic acid purification instrument, *ExiPrep*™ 16 Plus.

The ***ExiPrep***™ **Plus Tissue Genomic DNA Kit** is suitable for the extraction of genomic DNA from animal tissues using the automatic nucleic acid purification instrument, ExiPrep™ 16 Plus. The protocol requires a sample disruption step with proteinase K in the supplied tissue lysis buffer for optimal extraction of genomic DNA.

The ***ExiPrep***™ **Plus Cell Genomic DNA Kit** is suitable for the extraction of genomic DNA from cultured cells using automatic nucleic acid purification instrument, *ExiPrep*™ 16 Plus. Collected cells must be resuspended in the provided resuspension buffer for optimal extraction of genomic DNA.

The ***ExiPrep***™ **Plus Bacteria Genomic DNA Kit** is suitable for the extract of genomic DNA from gram negative bacteria, gram positive bacteria and yeast using the automatic nucleic acid purification instrument, *ExiPrep*™ 16 Plus. Gram positive bacteria and yeast need enzymatic digestion step with lyticase or lysozyme to make spheroplasts. After pretreatment with those enzymes, the prepared spheroplasts must be resuspended in the provided resuspension buffer for optimal extraction of genomic DNA. The detailed experimental protocol is described on page 11.

The ***ExiPrep***™ **Plus Plant Genomic DNA Kit** is suitable for the extraction of genomic DNA from plant tissue or seeds using the automatic nucleic acid purification instrument, *ExiPrep*™ 16 Plus. The protocol requires a sample disruption step with proteinase K in the supplied plant lysis buffer for optimal extraction of genomic DNA.

III. Storage

ExiPrep™ Plus Genomic DNA Kits utilize our unique Buffer cartridge system. The Buffer cartridges contain all components for nucleic acid extraction, including: binding buffer, washing buffer, elution buffer and magnetic bead solution. Each Buffer cartridge is hermetically sealed with a three-ply sealing foil and then wrapped in film to protect against leakage, evaporation and cross-contamination. The Buffer cartridges can be stored dry at room temperature (15℃-25℃) for up to 2 years from the date of delivery, provided they remain sealed.

ExiPrep™ Plus Genomic DNA Kits also contain lyophilized enzymes (proteinase K and RNase A), where applicable, for your convenience. Lyophilized enzymes are pre-loaded into Buffer cartridges (RNase A) or 2.0 ml screw cap tubes (proteinase K). They can be stored at room temperature (15℃-25℃) up to 2 years without any reduction in activity provided they remain unopened. Once dissolved, enzymes should be stored at -20℃ for up to 6 months.

All provided consumables, including disposable tips, reaction tubes and elution tubes, are DNase- and RNase-free.

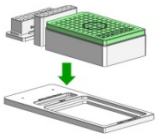
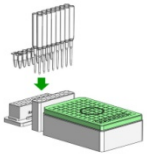
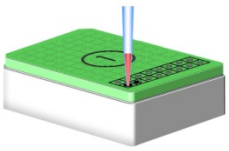
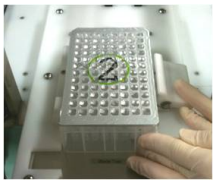
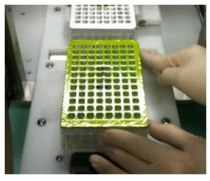
IV. Starting volume

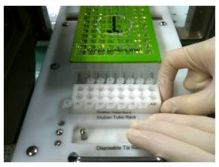
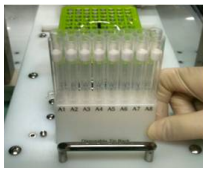
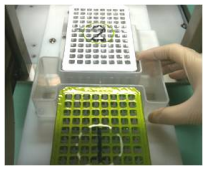
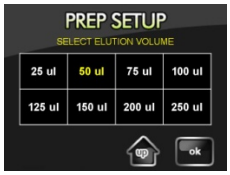
The starting amount (volume or weight), elution volume and the typical yields of extracted genomic DNA are described in below.

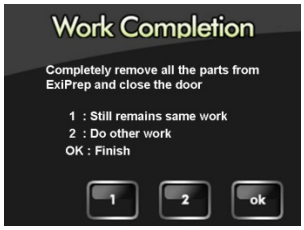
Sample type	Starting Amount	Elution Volume	Typical Yield
Whole blood (Healthy human)	200 µl	50–200 µl	2–5 µg
Animal tissue (Bovine muscle)	10–40 mg	50–200 µl	5–10 µg
Animal tissue (Rat tail tip)	0.5 cm	50–200 µl	5–10 µg
Cultured cells (HeLa cells)	~1x10^6 cells	50–200 µl	5–15 µg
Gram (–) bacteria	~1x10^9 cells	50–200 µl	5–15 µg
Gram (+) bacteria	~1x10^9 cells	50–200 µl	5–15 µg
Yeast (*S. pombe*)	~1x10^9 cells	50–200 µl	5–15 µg
Yeast	~1x10^9 cells	50–200 µl	5–15 µg
Plant tissue (Fresh leaf tissue)	100 mg	50–200 µl	0.25–5 µg
Plant seed (Bean)	20–30 mg	50–200 µl	1–5 µg

V. Genomic DNA extraction from whole blood

This protocol is designed for extraction of Genomic DNA from whole blood, buffy coat, urine or any liquid phase sample

	1. Puncture the cartridges with the hole-punch tool to correspond with the sample number that will be used (1-16). Before punching the hole, agitate the Buffer cartridge gently to settle the beads and buffer.
	2. Place the Buffer Cartridge ①, Elution tube rack and Disposable tip rack on the setup tray.
	3. Load the Disposable filter tips and Elution tubes onto the racks. If using less than 16 samples, make sure that all tips and tubes are aligned in desired position.
	4. Load 200 µl of sample into the sample loading wells. Make sure to follow any pre-treatment steps as described in sections V-IX (depending on sample type). Take care to avoid contaminating any other wells.
	5. Place the Buffer Cartridge ② onto the proper position in the rear of the base plate. Check that the punched holes of Buffer Cartridge ② match Buffer Cartridge ①.
	6. Place the Buffer Cartridge ① onto the proper position of the base plate. Check that the punched holes of the Buffer Cartridge ① match Buffer Cartridge ②.

	7. Place the Elution tube rack onto the proper position of the base plate. The elution rack is slotted so it can only be placed in the correct orientation.
	8. Place the Disposable filter tip rack onto the proper position of the base plate.
	9. Place the Waste tray onto the proper position on the base plate between Buffer Cartridge ① and Buffer Cartridge ②. 10. Push the base plate back into the instrument and close the door.
	11. Turn on the *ExiPrep*™ 16 Plus. 12. Press the 'Start' button to access the PREP SETUP menu.
	13. Input a protocol number according to the protocol number list (Page 14). 14. Press the 'Enter' button to move to the next step.
	15. Select the desired elution volume from the touch screen. 16. Press the 'ok' button to move to the next step.
	17. Verify the loaded every racks and buffer cartridges in the correct position on the base plate according to the 'CHECK LIST' like as followings.
	18. Verify the protocol name on the screen. 19. Press the 'Run' button to start an extraction run.

	20. After Protocol completion, open the door and take the Elution tube from base plate. 21. Remove the remaining accessories from the base plate and close the door.

VI. Genomic DNA extraction from animal tissue

This protocol is designed for extraction of Genomic DNA from animal tissues (muscle, liver, kidney, spleen, heart, tail, etc.).

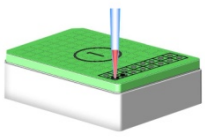
When ready to start, dissolve the proteinase K (20 mg) into 1.0 ml of DNase- and RNase-free water.

Note that this protocol requires a shaking water bath and table top centrifuge.

Tissue lysis buffer may form precipitates during storage. If this occurs, please warm the tissue lysis buffer to 60℃ until the precipitates are completely dissolved.

Disrupt tissues according to step A, step B or step C.

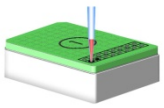
A. Tissue samples can be disrupted before proteinase K digestion by pestle & mortar or any tissue disruptor with or without liquid nitrogen.

	1. Add liquid nitrogen into the mortar to cool the mortar and pestle. 2. Cut up to 10-40 mg of animal tissue, and transfer that sample into the mortar. 3. Make a fine powder with the pestle.
	4. Transfer the powdered tissue into 1.5 ml test tube (not provided). 5. Add 20 µl of Proteinase K (20 mg/ml) and 200 µl of Tissue lysis buffer into that 1.5ml test tube.
	6. Incubate the tube at 60℃ for at least 2 hr with shaking. 7. Centrifuge the tube at 13,000 rpm for 5 min to remove any unlysed tissue.
	8. Take the supernatant only and transfer into the new 1.5 ml test tube (not provided). 9. Go to step 1. of the 'Genomic DNA extraction from whole blood' (page 5).

B. Tissue samples may be disrupted by overnight digestion with proteinase K.

	1. Cut up to 10–40 mg of animal tissue and transfer into the 1.5 ml test tube (not provided). 2. Add 20 µl of proteinase K (20 mg/ml) and 200 µl of Tissue lysis buffer into that 1.5 ml test tube. 3. Incubate the tube at 60℃ overnight with shaking. 4. Go to step 1. of the 'Genomic DNA extraction from whole blood' (page 5).

C. Tissue samples may be homogenized with Bioneer's tissue homogenization set (Cat. No. A-7030) before proteinase K digestion.

	1. Cut up to 10–40 mg of animal tissue and transfer into the tissue filter tube. 2. Add 200 µl of Tissue lysis buffer. 3. Briefly grind the tissue with tissue homogenizer for 30 sec.
	4. Make sure to completely disrupt the tissue with Tissue homogenizer. Complete homogenization provides excellent lysis efficiency and improved yield.
	5. Centrifugation the filter tube at 13,000 rpm, for 5 min in a table top centrifuge. 6. Add 20 µl of proteinase K (20 mg/ml) into a new 1.5 ml test tube.
	7. Transfer the filtrate into the new 1.5 ml test tube and mix well by vortexing. 8. Go to step 1. of the 'Genomic DNA extraction from whole blood' (page 5).

VII. Genomic DNA extraction from cultured cell

This protocol is designed for extraction of Genomic DNA from cultured cells.

Resuspension buffer may form a precipitate during storage. Should this occur, please warm to 60℃ until the precipitate is completely dissolved.

Cell harvest:

- for cells grown in suspension
  Determine the number of cells using hemocytometer. Pellet the appropriate number of cells (~1x10^6) by centrifugation at 3,000 rpm for 5min. Remove the supernatant, wash the pellet with sterile 1X PBS, and re-centrifuge to pellet. Then follow steps 1 & 2 below.

- for cells grown in a monolayer
  Cells can be either lysed directly in the cell-culture vessel or trypsinized and collected as a cell pellet prior to lysis.

  · *To lyse cells directly*
  Determine the number of cells. Aspirate the culture medium completely. Then follow steps 1 & 2 below.

  · *To trypsinize and collect cells*
  Determine the number of cells. Aspirate the culture medium, and wash the cells with sterile 1X PBS. Aspirate the PBS, and add trypsin-EDTA solution in 1X PBS. After the cells detach from the culture vessel, add medium containing serum to inactivate the trypsin. Then, transfer the cells to a centrifuge tube and centrifuge at 3,000 rpm for 5min. Aspirate the supernatant. Then follow steps 1 & 2 below.

	1. Resuspend the cell pellet or monolayer (up to 1×10^6 cells) in 200 µl of Resuspension buffer. 2. Go to Step 1. of the 'Genomic DNA Extraction from Whole blood' (page 5).

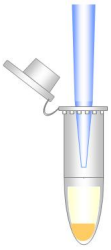
VIII. Genomic DNA extraction from bacteria

This protocol is designed for extraction of Genomic DNA from Gram negative and Gram positive bacteria.

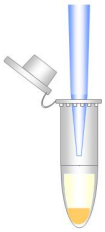
ExiPrep™ 16 Plus Bacteria Genomic DNA Kit requires enzymatic lysis for cell wall disruption of gram positive bacteria with lysozyme (not provided in the kit).

Resuspension buffer may form a precipitate during storage. Should this occur, please warm to 60℃ until the precipitate is completely dissolved.

A. Protocol for Gram negative bacteria.

	1. Resuspend the cell pellet (up to 1×10^9 cells) in 200 µl of Resuspension buffer. 2. Go to Step 1. of the 'Genomic DNA Extraction from Whole blood' (page 5).

B. Protocol for Gram positive bacteria.

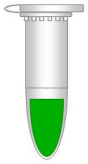
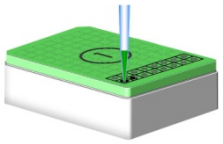
	1. Resuspend the cell pellet (up to 1×10^9 cells) in 200 µl of 1X TE buffer. 2. Add 20 µl of lysozyme (50 mg/ml) and incubate the tube at 37℃ for at least 1 hr to form spheroplasts. (Note: If using Zymolase for yeast, add to a final concentration of 50 U per 1×10^6 cells with 0.1% of β-mercaptoethanol and incubate for 30 min at 30 ℃)
	3. Centrifuge the tube at 13,000 rpm for 5 min in a microcentrifuge. 4. After the centrifugation, discard the supernatant by pipetting and add 200 µl of Resuspension buffer and mix well. 5. Go to Step 1. of the 'Genomic DNA Extraction from Whole blood' (page 5).

IX. Genomic DNA extraction from plant

This protocol is designed for the extraction of Genomic DNA from plant tissue (leaf, stalk, root, flower, etc.) and seeds.

Before beginning dissolve the proteinase K (20 mg) into 1.0ml of DNase- and RNase-free water.

This protocol requires a shaking water bath and microcentrifuge.

	1. Add 20 µl of proteinase K (20 mg/ml) into the 1.5 ml test tube (not provided). 2. Add 100 mg (fresh) or 10-20 mg (dry) of plant into mortar and grind into a powder with liquid nitrogen. 3. Transfer the powdered plant into the 1.5 ml tube containing proteinase K. 4. Add 300 µl of Plant Lysis buffer.
	5. Incubate the tube at 60℃ for at least 1 hr with shaking. 6. Centrifuge the tube at 13,000 rpm for 5 min to remove any unlysed tissue.
	7. Take the supernatant only and transfer into the new 1.5 ml test tube (not provided). 8. Go to step 1. of the 'Genomic DNA extraction from whole blood' (page 5).

※ Plant tissue or seeds can be disrupted with mechanical bead-based methods instead of liquid nitrogen.

X. Troubleshooting

1. Low yield of Genomic DNA

1) Did you add too much (or too little) sample? The yield is dependent on the sample type and amount. Too much or too little sample will decrease yields.

2) Did you completely lysis the samples? Did you completely clear the lysate via centrifugation? Incomplete lysis and clearing decreases the yield and purity.

3) Did you agitate Buffer cartridge ① before use? Incomplete suspension of the magnetic bead may decrease the yield and purity.

2. Co-eluted magnetic particle

Sometimes magnetic particles are carried-over with your Genomic DNA after elution. Carryover of magnetic particles in the eluate will not affect the performance of the gDNA in downstream applications. Furthermore, magnetic particle cannot bind Genomic DNA in elution buffer, though it may affect readings on a spectrophotometer.
Magnetic particles that are carried over can be easily separated by centrifugation for 1 min at 13,000 rpm in a microcentrifuge.

XI. Additional protocols

1. Genomic DNA Extraction from FFPE tissue

a. Transfer the 1 piece of sectioned FFPE tissue into a 1.5 ml test tube.
b. Add 1 ml of xylene and vortex for 30 sec.
c. Centrifuge the tube at 13,000 rpm room temp. for 5 min and remove the xylene by pipetting.
d. Add 1 ml of absolute ethanol and vortex for 30 sec.
e. Centrifuge the tube at 13,000 rpm room temp. for 5 min and remove the ethanol by pipetting.
f. Repeat step d. and e.
g. Dry the tissue at 60℃ in a heating block or oven to completely evaporate the residual ethanol.
h. Go to step B. of the **VI. Genomic DNA Extraction from Animal tissue** in page 9.

2. Genomic DNA Extraction from yeast

a. Collect the yeast cells (~1x10^9 cells) into the 1.5 ml test tube.
b. Add 1 ml of 1X PBS and vortex for 30 sec.
c. Centrifuge the tube at 13,000 rpm room temp. for 5 min and remove the 1X PBS by pipetting.
d. Go to step 1. of the '**Protocol for Gram positive bacteria**' in page 11, and follow the digestion step.

XII. List of protocol numbers

No.	Target	Sample source
1 01	Genomic DNA	Whole blood
1 02	Genomic DNA	Animal tissue
1 03	Genomic DNA	FFPE tissue
1 04	Genomic DNA	Plant tissue
1 05	Genomic DNA	Plant seed
1 06	Genomic DNA	Rice
1 07	Genomic DNA	Cultured cell
1 08	Genomic DNA	Gram (+) bacteria
1 09	Genomic DNA	Gram (−) bacteria
1 10	Genomic DNA	Yeast
1 11	Genomic DNA	Yeast
1 14	Genomic DNA	Buffy coat
1 15	Genomic DNA	Sputum
1 16	Genomic DNA	BAL
1 17	Genomic DNA	Saliva
1 18	Genomic DNA	Swab
1 19	Genomic DNA	Urine
1 10	Genomic DNA	Stool
1 23	Genomic DNA	CSF
1 24	Genomic DNA	EPS
1 25	Genomic DNA	Respiratory sample
1 26	Genomic DNA	Amniotic fluid
1 27	Genomic DNA	Forensic sample
1 28	Genomic DNA	Bone marrow
1 29	Genomic DNA	Bone
1 30	Genomic DNA	Dried blood spot
1 31	Genomic DNA	Soil
1 32	Genomic DNA	Hair
1 33	Genomic DNA	Cell supernatant

XIII. Explanation of symbols

Catalog Number

Sufficient Reagents for X tests

Manufacturer

XIV. Ordering Information

Product	Size	Cat. No.
ExiPrep™ 16 Plus	1 ea	A-5030
ExiPrep™ *Plus* Blood Genomic DNA Kit	96 preps.	K-4211
ExiPrep™ *Plus* Tissue Genomic DNA Kit	96 preps.	K-4212
ExiPrep™ *Plus* Bacteria Genomic DNA Kit	96 preps.	K-4214
ExiPrep™ *Plus* Plant Genomic DNA Kit	96 preps.	K-4215
ExiPrep™ *Plus* Beef Genomic DNA Kit	96 preps.	K-4216
ExiPrep™ *Plus* Rice Genomic DNA Kit	96 preps.	K-4217
ExiPrep™ *Plus* Tissue Total RNA Kit	96 preps.	K-4242
ExiPrep™ *Plus* Plant Total RNA Kit	96 preps.	K-4244
ExiPrep™ *Plus* Viral DNA Kit	96 preps.	K-4272
ExiPrep™ *Plus* Viral RNA Kit	96 preps.	K-4273
ExiPrep™ *Plus* Viral DNA/RNA Kit	96 preps.	K-4271

Bioneer Worldwide

Headquarters

Address 8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon 306-220, South Korea
Tel +82-42-930-8777 (Korea : 1588-9788)
Fax +82-42-930-8600
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.us.com
Web www.bioneer.com

Bioneer Trade(Shanghai) Co., Ltd

Address 403 Room, 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 salescn@bioneer.com
Web www.bioneercn.com

Seoul Office

Address 2nd fl. Sansu B/D, #66, Gangnam-daero 30-gil, Seocho-gu, Seoul, 137-130, South Korea
Tel +82-2-598-1094 (Korea : 1588-9788)
Fax +82-2-598-1096
E-mail sales@bioneer.co.kr
Web http: //www.bioneer.co.kr

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