USER'S GUIDE

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ExiPrep™ Plus Genomic DNA Kit

REF K-4211
K-4214
K-4215
K-4217

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ExiPrep™ Plus Blood Genomic DNA Kit (K-4211)
ExiPrep™ Plus Bacteria Genomic DNA Kit (K-4214)
ExiPrep™ Plus Plant Genomic DNA Kit (K-4215)
ExiPrep™ Plus Seed Genomic DNA Kit (K-4217)

User's Guide

Version No.: 5.0 (2018-11-09)

Please read all the information in booklet before using the unit

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Intended Use

ExiPrep™ Plus Genomic DNA Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

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 Sheet (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.

Trademark

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Contents

I. Kit components

	ExiPrep™ Plus Genomic DNA Kit			
Cat. No	Blood (K-4211)	Bacteria (K-4214)	Plant (K-4215)	Seed (K-4217)
Buffer Cartridge ①	6 ea	6 ea	6 ea	6 ea
Buffer Cartridge ②	6 ea	6 ea	6 ea	6 ea
Resuspension Buffer		1 ea		
Plant Lysis Buffer	-	-	1 ea	-
Seed Lysis Buffer	-	-	-	1 ea
Precipitation Buffer	-	-	-	1 ea
Proteinase K (20mg)	-	-	2 ea	2 ea
RNase A (24mg)	-	-	-	2 ea
Disposable Filter Tip	96 ea	96 ea	96 ea	96 ea
Elution Tube (8-strip)	12 ea	12 ea	12 ea	12 ea
User's Guide	1 ea	1 ea	1 ea	1 ea

II. Introduction

***ExiPrep*™ Plus Blood Genomic DNA Kit** is suitable for the extraction of genomic DNA from whole blood using the automatic nucleic acid purification instrument, *ExiPrep*™ 16 Plus.

***ExiPrep*™ Plus Plant Genomic DNA Kit** is suitable for the extraction of genomic DNA from plant tissue 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.

***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.

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

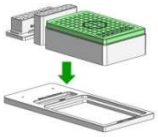
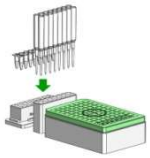
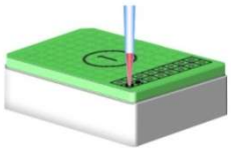
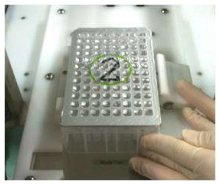
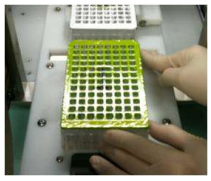
All provided consumables, including disposable tips, reaction tubes and elution tubes, are DNase- and RNase-free.

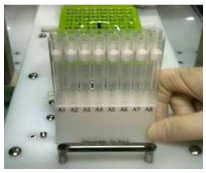
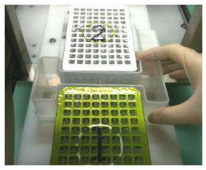
IV. Starting amount and typical yield

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	~ 200 µl	50 – 150 µl	1 – 5 µg
Cultured cells (HeLa cells)	~ 10^6 cells	50 – 150 µl	5 – 15 µg
Gram (–) bacteria	~ 10^9 cells	50 – 150 µl	5 – 15 µg
Gram (+) bacteria	~ 10^9 cells	50 – 150 µl	5 – 15 µg
Yeast (*S. pombe*)	~ 10^9 cells	50 – 150 µl	5 – 15 µg
Yeast	~ 10^9 cells	50 – 150 µl	5 – 15 µg
Plant tissue (Fresh leaf tissue)	~ 100 mg	50 – 150 µl	0.25 – 5 µg
Plant seed	~ 50 mg	50 – 150 µl	0.25 – 5 µg

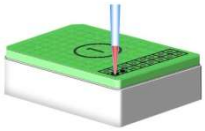
V. Loading the kit to the instrument and Genomic DNA extraction

	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 – 300µl of sample into the sample loading wells. Make sure to follow any pre-treatment steps as described in sections VI-X (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 whole blood

This protocol is designed for extraction of Genomic DNA from whole blood or buffy coat.

	1. If the sample volume is less than 200 µl, bring the sample volume to a total volume of 200 µl by adding 1X PBS to achieve maximum lysis efficiency and yield.
	2. Go to step 1. of the 'Kit loading the instrument and Genomic DNA extraction' (page 5).

VII. Genomic DNA extraction from cultured cell

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

Cell harvest:

- for cells grown in suspension

  Determine the number of cells using hemocytometer. Pellet the appropriate number of cells (~ 1 x 10^6) by centrifugation at 3,000 rpm for 5 min. 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 3,000rpm 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 1X PBS. 2.Go to Step 1. of the 'Kit loading the instrument and Genomic DNA extraction' (page 5).

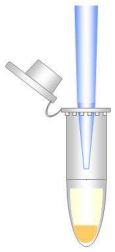
VIII. Genomic DNA extraction from bacteria

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

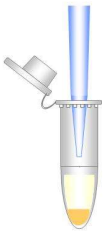
ExiPrep™ Plus Bacteria Genomic DNA Kit requires enzymatic lysis for cell wall disruption of gram positive bacteria with lysozyme (not provided in the kit).

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 x 10^9 cells) in 200 µl of Resuspension buffer. 2. Go to Step 1. of the 'Kit loading the instrument and Genomic DNA extraction' (page 5).

B. Protocol for Gram positive bacteria.

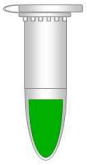
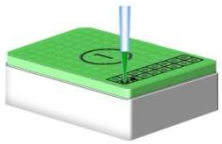
	1.Resuspend the cell pellet (up to 1 × 10^9 cells) in 200 µl of 1X TE buffer. 2.Add 20 µl of lysozyme (50 mg/ml) and incubate the tube at 37℃ for at least 1 hr to form spheroplasts. (Note: If using Zymolase for yeast, add to a final concentration of 50 U per 1×10^6 cells with 0.1% of β-mercaptoethanol 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 'Kit loading the instrument and Genomic DNA extraction' (page 5).

IX. Genomic DNA extraction from plant tissue

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

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

This protocol requires 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 30 min – 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 'Kit loading the instrument and Genomic DNA extraction' (page 5).

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

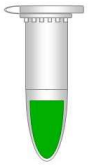
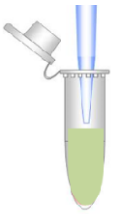
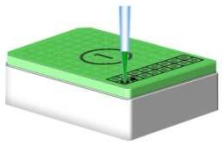
X. Genomic DNA extraction plant (GMO) seeds

This protocol is designed for the extraction of Genomic DNA from plant (GMO) seeds.

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

Before beginning dissolve the RNase A (24 mg) into 600 µl of DNase- and RNase-free water.

This protocol requiresshaking water bath and microcentrifuge.

	1. Add 50 mg (fresh) or 10 – 20 mg (dry) of plant into mortar and grind into a powder with liquid nitrogen. 2. Transfer the powdered plant into the 1.5 ml tube (not provided).
	3. Add 20 µl of proteinase K (20 mg/ml) and 10 µl of RNase A (40mg/ml) into the 1.5 ml test tube. 4. Add 270 µl of Seed Lysis buffer. 5. Incubate the tube at 60℃ for at least 10min.
	6. Add 90 µl of Precipitation Buffer. 7. Incubate the tubes on ice for 5 min. 8. Centrifuge the tube at 13,000 rpm for 5 min to remove any precipitates.
	9. Take the supernatant only. 10. Load 300 µl of supernatant into the sample loading wells of Buffer Cartridge ①. 11. Go to step 1. of the 'Kit loading the instrument and Genomic DNA extraction' (page 5).

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

XI. 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 genomic DNA 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.

XII. Additional protocol

1. Genomic DNA Extraction from yeast

a. Collect the yeast cells (~1 x 10^9 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 temperature for 5 min and remove the 1X PBS by pipetting.

d. Resuspend the cell pelletin 200 µl of 1X TE buffer.

e. Add 20 µl of lysozyme (50mg/ml) and incubate the tube at 37℃ for at least 1 hr. (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 ℃.)

f. Centrifuge the tube at 13,000 rpm for 5 min in a microcentrifuge.

g. After the centrifugation, discard the supernatant by pipetting and add 200 µl of Resuspension buffer and mix well

h. Go to Step 1. of the 'Kit loading the instrument and Genomic DNA extraction' (page 5).

XIII. 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

XIV. Explanation of symbols

REF	Catalog Number	Σ	Contains sufficient for (n) tests		USE BY
LOT	Batch code	⚠	Caution, consult accompanying documents		Temperature Limitation
	Manufacturer		Caution, Potential Biohazard		DO NOT REUSE
	Consult Instruction For Use				

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