ISO 9001
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Ver. 2.0

User's Guide

Global Genomics Partner

ExiPrep™ Genomic DNA Kit

ExiPrep™ **Blood Genomic DNA Kit (K-3215)**
ExiPrep™ **Tissue Genomic DNA Kit (K-3225)**
ExiPrep™ **Cell Genomic DNA Kit (K-3235)**
ExiPrep™ **Bacteria Genomic DNA Kit (K-3245)**
ExiPrep™ **Plant Genomic DNA Kit (K-3255)**

BIONEER
bioneer corporation

Safety Warnings and Precautions

ExiPrep™ Genomic DNA Kit is developed and sold for research purposes 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.

Quality Management System ISO 9001 Certified

Every aspect of Bioneer's quality management system from product development to production to quality assurance and supplier qualification meets or exceeds the world-class quality standards.

Trademarks

ExiPrep™ is trademark of Bioneer Corporation.

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I. Kit Components

	ExiPrep™ Genomic DNA Kit				
Cat. No	Blood (K-3215)	Tissue (K-3225)	Cell (K-3235)	Bacteria (K-3245)	Plant (K-3255)
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	-	-	-	-	2 ea
Proteinase K (20.0mg)	-	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*™ Blood Genomic DNA Kit is suitable to extract of genomic DNA from whole blood, buffy coat, urine and any liquid phase sample. The *ExiPrep*™ Tissue Genomic DNA Kit is suitable to extract of genomic DNA from animal tissue, it needs sample disruption step with proteinase K and optimized Tissue lysis buffer for the genomic DNA extraction. The *ExiPrep*™ Cell Genomic DNA Kit is suitable to extract of genomic DNA from cultured cell. Collected cells must resuspend with provided Resuspension buffer for the genomic DNA extraction instead of PBS buffer. The *ExiPrep*™ Bacteria Genomic DNA Kit is suitable to extract of genomic DNA from gram negative bacteria, gram positive bacteria and fungi. Gram positive bacteria and fungi need enzymatic digestion step with lyticase or lysozyme to make a spheroplast cell. After pretreatment with those enzymes, prepared spheroplast cells must resuspend with provided Resuspension buffer for the genomic DNA extraction. More detailed experimental protocol is described in page 11. *ExiPrep*™ Plant Genomic DNA Kit is suitable to extract of genomic DNA from plant tissue and seed, it needs sample disruption step with proteinase K and optimized Plant lysis buffer for the genomic DNA extraction. And supplied two types of lysis buffer were optimized to its sample resources. Plant lysis buffer 1 is optimized to plant leaf tissue sample and Plant lysis buffer 2 is optimized to seed sample.

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III. Storage

ExiPrep™ Genomic DNA Kits provide Buffer cartridge system. The Buffer cartridges contains binding buffer, washing buffer, elution buffer and magnetic bead solution for the nucleic acid extraction. Every Buffer cartridges were covered with sealing film to protect leakage, evaporation and cross contamination. The Buffer cartridges can be stored dry at room temperature (15℃-25℃) for up to 2 years without open.

ExiPrep™ Genomic DNA Kits provide lyophilized enzymes (proteinase K, RNase A...) for the convenient use.
Lyophilized enzymes (proteinase K, RNase A...) were pre-loaded into Buffer cartridges and 2.0ml screw cap tubes. It can be stored at room temperature (15℃-25℃) up to 2 years without any reduced activity. And dissolved enzymes must be stored at -20℃ for longer storage.

Provided disposable tips, reaction tubes and elution tubes are DNase and RNase free, please give attention to the nuclease contamination during storing.

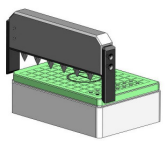
IV. Starting Volume and Typical Yield

■ The amounts of starting volume, elution volume and the typical yields are described in below.

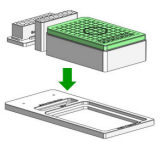
Sample type	Starting Volume	Elution Volume	Typical Yield
Whole blood (Healthy human)	200ul	50-200ul	2-5ug
Animal tissue (Bovine muscle)	10-40mg	50-200ul	5-10ug
Animal tissue (Rat tail tip)	0.5cm	50-200ul	5-10ug
Cultured cells (HeLa cells)	~1x10^{6}cells	50-200ul	5-15ug
Gram (-) bacteria	~1x10^{9}cells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

V. Genomic DNA Extraction from Whole blood

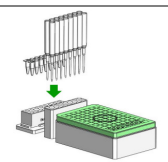
■ This protocol is designed for extraction of Genomic DNA from whole blood, buffy coat, urine and any liquid phase sample.



1. Make holes with the hole-punch tool to correspond with the sample numbers.
   Before punching the hole, shake the Buffer cartridge gently to settle down the bead and buffers.



2. Place the Buffer Cartridge ①, Elution tube rack and Disposable tip rack on the setup tray.

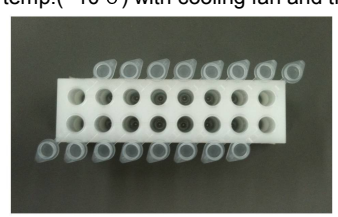


3. Load the Disposable filter tips and Elution tubes onto the racks. Ensure that all tips and tubes are aligned in desired position.

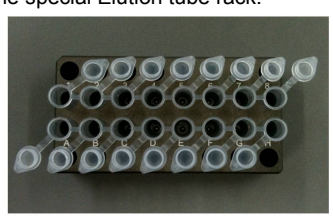
※ **Caution!!!**

ExiPrep™ 16 Plus & Pro provides different types of Elution tube rack for its special purposes. Please check the Elution tube's direction and position as described below.

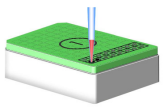
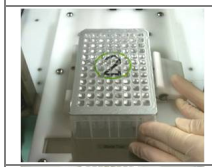
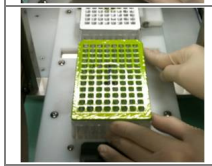
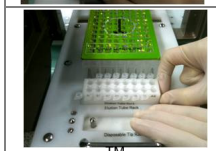
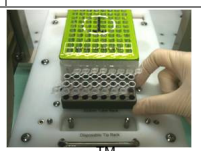
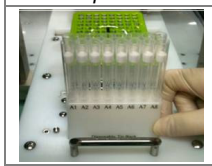
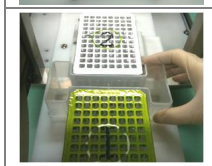
ExiPrep™ 16 Pro provides nucleic acid storage block at lower temp.(~10℃) with cooling fan and the special Elution tube rack.

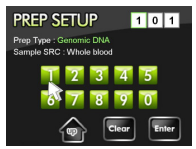
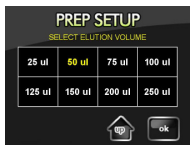
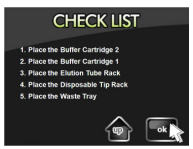
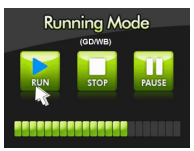


Elution tube & Elution tube rack for the *ExiPrep*™ 16 Plus



Elution tube & Elution tube rack for the *ExiPrep*™ 16 Pro

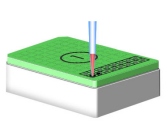
	4. Load 200ul of sample into the sample loading wells. Do not contaminate another wells.
	5. Place the Buffer Cartridge ② onto the proper position of the base plate. Please check the punched holes of the Buffer Cartridge ②.
	6. Place the Buffer Cartridge ① onto the proper position of the base plate. Please check the punched holes of the Buffer Cartridge ①.
 *ExiPrep*™ 16 Plus  *ExiPrep*™ 16 Pro	7. Place the Elution tube rack onto the proper position of the base plate.
	8. Place the Disposable filter tip rack onto the proper position of the base plate.
	9. Place the Waste tray onto the proper position of 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/ Pro 12. Press the 'Start' button to access the PREP SETUP menu.
	13. Insert a protocol number according to the protocol number list(Page 15) about nucleic acid types and sample sources. 14. Press the 'Enter' button to move to the next step.
	15. Select a 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. The first two letters represent a type of nucleic acid you will purify, and the next two letters represent a sample source. 19. Press the 'Run' button to start an extraction run.
	20. After the completion of the Instrument's operation, take the Elution tube from base plate first. 21. Remove the buffer cartridges, each racks and Waste tray 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...).
- At the beginning, dissolve the proteinase K (20mg) into 1.0ml of DNase, RNase free water.
- This protocol required shaking water bath and table top centrifuge.
- Tissue lysis buffer may form precipitates during storage. Please warm to 60℃ until the precipitates 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 and any tissue disruptor with liquid nitrogen or any tissue disruptor.

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

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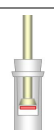
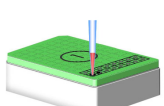
B. Tissue samples can be disrupted with proteinase K digestion for O/N.

	a. Cut up to 10-40mg of animal tissue and transfer into the 1.5ml test tube(not provided). b. Add 20ul of proteinase K and 200ul of Tissue lysis buffer into the 1.5ml test tube. c. Go to step 6. of the 'Genomic DNA extraction from Animal tissue'(page 8).

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

	1. Cut up to 10-40mg of animal tissue and transfer into the tissue filter tube. 2. Briefly grind the tissue with tissue homogenizer for 30 sec. 3. Add 200ul of Tissue lysis buffer.
	4. Disrupt the tissue with Tissue homogenizer completely. Complete homogenization will provide high lysis efficiency.
	5. Centrifugation the filter tube at 13,000 rpm, for 5 min. in a table top centrifuge. 6. Add 20ul of proteinase K into the new 1.5ml test tube.
	7. Transfer the filtrate into the 1.5ml 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 cell.
- Resuspension buffer may form precipitates during storage. Please warm to 60℃ until the precipitates completely dissolved.
- Cell harvest method

- Cells grown in suspension

  Determine the number of cells. Pellet the cells by centrifuge at 3,000rpm for 5min. Remove the supernatant and wash the pellet with sterile 1X PBS.

- 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.

· 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.

	1. Resuspend the cell pellet(up to 1× 10^6 cells) in 200ul 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 positive bacteria.
- *ExiPrep*™ Bacteria Genomic DNA Kit recommends enzymatic lysis for cell wall disruption of the gram positive bacteria with lysozyme. (not provided)
- Resuspension buffer may form precipitates during storage. Please warm to 60℃ until the precipitates completely dissolved.

A. Protocol for Gram negative bacteria.

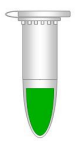
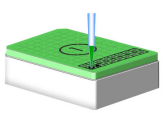
	1. Resuspend the cell pellet(up to 1× 10^6 cells) in 200 ul of Resuspension buffer. 2. Go to Step 1. of the 'Genomic DNA Extraction from Whole blood'(page 5).

B. Protocol for Gram positive bacteria.

	1. Resuspend the cell pellet(up to 1× 10^6 cells) in 200 ul of 1X TE buffer. 2. Add 20 ul of lysozyme(50mg/ml) and incubate the tube at 37℃ for at least 1 hr.
	3. Centrifugation the tube at 13,000 rpm, for 5 min. in a table top centrifuge. 4. After the centrifugation, discard the supernatant by pipetting and add 200 ul 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 extraction of Genomic DNA from plant tissue(leaf, stalk, root, flower...) and seed.
- At the beginning dissolve the proteinase K(20mg) into 1.0ml of DNase, RNase free water.
- This protocol required shaking water bath and table top centrifuge.

	1. Add 20ul of proteinase K into the 1.5ml test tube. 2. 100mg(fresh) or 10-20mg(dry) of plant into mortar and grind with liquid nitrogen. 3. Transfer the powdered plant into the tube. 4. Add 300ul of Plant Lysis buffer.(Plant leaf sample : Lysis buffer 1, Other plant sample : Lysis buffer 2)
	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 the incompletely lysed tissue.
	7. Take the supernatant only and transfer into the new 1.5ml test tube(not provided). 8. Go to step 1. of the 'Genomic DNA extraction from whole blood'(page 5).

※ Plant tissue and seed can be disrupted with mechanical disruption method using iron bead instead of liquid nitrogen.

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X. Troubleshooting

1. Low yield of Genomic DNA

1) Did you add sufficient amount of samples? The yield is dependent on the sample type and amount. Sometimes overload sample may decrease the yield.

2) Did you completely lysis the samples? Incomplete lysis decreases the yield and purity.

3) Is there precipitated salt in the Tissue lysis buffer, Plant lysis buffer and Resuspension buffer? Keep the bottles at 60℃ to re-dissolve.

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

2. Co-eluted magnetic particle

Sometimes magnetic particle co-eluted with your extracted DNA after genomic DNA extraction. Co-eluted magnetic particle can not bind DNA and RNA in elution buffer and it will not decrease the yield and purity.
Co-eluted magnetic particles can easily separate by simple centrifugation.

XI. Additional Protocol

1. Genomic DNA Extraction from FFPE tissue

a. Transfer the 1 piece of sectioned FFPE tissue into 1.5ml test tube.
b. Add 1ml 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 1ml 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℃ dry oven for completely evaporate the residual ethanol.
h. Go to step 1. of the **VI. Genomic DNA Extraction from Animal tissue** in page 8.

2. Genomic DNA Extraction from fungi

a. Collect the fungi cells into 1.5ml test tube.
b. Add 1ml 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.

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XII. Protocol Number List

No.	Sample source
101	Whole blood
102	Animal tissue
103	FFPE tissue
104	Plant tissue
105	Plant seed
106	Rice
107	Cultured cell
108	Gram (+) bacteria
109	Gram (-) bacteria
110	Yeast
111	Fungi
114	Buffy coat
115	Sputum
116	BAL
117	Saliva
118	Swab
119	Urine
120	Stool
123	CSF
124	EPS
125	Respiratory sample
126	Amniotic fluid
127	Forensic sample
128	Bone marrow
129	Bone
130	Dried blood spot
131	Soil
132	Hair
133	Cell supernatant

XIII. Ordering Information

Product	Size	Cat. No.
ExiPrep™ 16 Plus	1 ea	A-5030
ExiPrep™ 16 Pro	1 ea	A-5040
ExiPrep™ Blood Genomic DNA Kit	96 prep.	K-3215
ExiPrep™ Tissue Genomic DNA Kit	96 prep.	K-3225
ExiPrep™ Cell Genomic DNA Kit	96 prep.	K-3235
ExiPrep™ Bacteria Genomic DNA Kit	96 prep.	K-3245
ExiPrep™ Plant Genomic DNA Kit	96 prep.	K-3255
ExiPrep™ Blood Total RNA Kit	96 prep.	K-3315
ExiPrep™ Tissue Total RNA Kit	96 prep.	K-3325
ExiPrep™ Cell Total RNA Kit	96 prep.	K-3335
ExiPrep™ Bacteria Total RNA Kit	96 prep.	K-3345
ExiPrep™ Plant Total RNA Kit	96 prep.	K-3355
ExiPrep™ Viral DNA Kit	96 prep.	K-3515
ExiPrep™ Viral RNA Kit	96 prep.	K-3525
ExiPrep™ Viral DNA/ RNA Kit	96 prep.	K-3535
ExiPrep™ Beef Genomic DNA Kit	96 prep.	K-3200-CB
ExiPrep™ Rice Genomic DNA Kit	96 prep.	K-3200-CR