USER GUIDE

ExiPrep™ Plus Genomic DNA Kit

Cat. No. 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)

Kit for the extraction of total DNA from whole blood, bacterial cells, plant tissues, or seeds

User Guide

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Version No.: 6 (2022-06-10)

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.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Patent

ExiPrep™ and its kits are protected by the patents KR10-2015-0089172.

Trademark

ExiPrep™ is a trademark of BIONEER Corporation.

Copyright

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

Components	K-4211 (Blood)	K-4214 (Bacteria)	K-4215 (Plant)	K-4217 (Seed)
Buffer Cartridge ①	6 ea	6 ea	6 ea	6 ea
Buffer Cartridge ②	6 ea	6 ea	6 ea	6 ea
Resuspension Buffer	-	25 ml x 1 ea	-	-
Plant Lysis Buffer	-	-	40 ml x 1 ea	-
Seed Lysis Buffer	-	-	-	30 ml x 1 ea
Precipitation Buffer	-	-	-	10 ml x 1 ea
Proteinase K powder, lyophilized	-	-	20 mg x 2 ea	20 mg x 2 ea
RNase A powder, lyophilized	-	-	-	24 mg x 2 ea
Disposable Filter Tip	3 packs (32 ea/pack)			
Elution Tube	8-tube strips x 12 ea			
User Guide	1 ea			

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

Storage

The kit will maintain performance for at least two years under standard storage conditions.

The Buffer Cartridges can be stored dry at room temperature (15-25°C) for up to 2 years from the date of delivery, provided they remain sealed.
The kit also contains lyophilized enzymes (Proteinase K and RNase A), which are pre-loaded into Buffer Cartridges (RNase A) or 2 ml screw cap tubes (Proteinase K). They can be stored at room temperature up to 2 years without any reduction in activity, provided they remain unopened. Once dissolved, enzymes should be stored at -20°C for up to 6 months.

Specifications

Sample Type	Amount of Starting Sample	Typical DNA Yield	DNA Purity
Whole blood	< 200 µl	1-5 µg	A_{260}/A_{280} > 1.8
Cultured cells (HeLa cells)	< 1 x 10^6 cells	5-15 µg	
Gram (-) bacteria cells	< 1 x 10^9 cells	5-15 µg	
Gram (+) bacteria cells	< 1 x 10^9 cells	5-15 µg	
Yeast (*S. pombe*)	< 1 x 10^9 cells	5-15 µg	
Yeast	< 1 x 10^9 cells	5-15 µg	
Plant tissue (Fresh leaf tissue)	< 100 mg	0.25-5 µg	
Plant seed	< 50 mg	0.25-5 µg	A_{260}/A_{280} > 1.7

*** Note:** There may be differences in measured values depending on the type of samples.

Precautions

The Buffer Cartridge ① and ② of this kit are covered with sealing film in order to prevent cross-contamination, evaporation, or leakage of solutions inside. All of the plastic products and buffers in this kit are provided under nuclease-free condition, hence, please be careful not to contaminate any part of the kit with nuclease.

Introduction

Product Description

ExiPrep™ Plus Genomic DNA Kits are designed for extraction of total DNA from whole blood, plant tissue, bacteria cells, or plant seeds. *ExiPrep*™ Plus Genomic DNA Kits provide total solution for accurate and rapid genomic DNA extraction. These kits employ our unique Buffer Cartridge system. The Buffer Cartridges contain all components for nucleic acid extraction, including: binding buffer, washing buffer, elution buffer, and magnetic nanobead solution. The Buffer Cartridges are key to extract total DNA with the aid of *ExiPrep*™16 Plus (Cat. No. A-5030). *ExiPrep*™16 Plus is designed for rapid extraction of nucleic acids delivering up to 16 extracted samples automatically.

Protocol of *ExiPrep*™ Plus Plant Genomic DNA Kit and *ExiPrep*™ Plus Seed Genomic DNA Kit requires a sample disruption step by using Proteinase K in the supplied Plant Lysis Buffer for optimal genomic DNA extraction.

For using *ExiPrep*™ Plus Bacteria Genomic DNA Kit, gram-positive bacteria and yeast need enzymatic digestion step with zymolyase or lysozyme to make spheroplasts. After pretreatment with those enzymes, the prepared spheroplasts should be resuspended in the provided resuspension buffer for optimal genomic DNA extraction.

The process does not require phenol/chloroform extraction and ethanol precipitation.
DNA extracted through this kit can be used for a variety of applications, including: gene cloning, PCR, Real-time PCR, genetics, and SNP genotyping.

Principle

ExiPrep™ Plus Genomic DNA Kits are designed for extraction of genomic DNA. These kits employ Magnetic Nano Beads coated with silica for nucleic acid binding in the presence of chaotropic salts. Guanidine hydrochloride as chaotropic agents in binding buffer removes water molecules around DNA and surface of the silica-coated magnetic nanobeads resulting in genomic DNA, which is captured by silica-coated magnetic nanobeads. The magnetic nanobeads and DNA complexes are pulled and fixed on the tube wall using a magnetic force. Any salts and precipitates are eliminated by washing buffer, and captured genomic DNA is eluted in an elution buffer or nuclease-free water.

Features & Benefits

- Comprehensive: High quality and yield of genomic DNA extraction from various samples.
- Convenient & Rapid: Uses a pre-filled buffer cartridge system in which enzymes and reagents for nucleic acid extraction are dispensed.
- Reproducible: Uses fully automatic nucleic acid extraction equipment, and reproducible results can be obtained.
- Efficient: Contains all required consumables such as Disposable Filter Tips and Elution Tubes.
- Ready-to-use: Extracted DNA is ready-to-use for various application.

Components of Buffer Cartridges

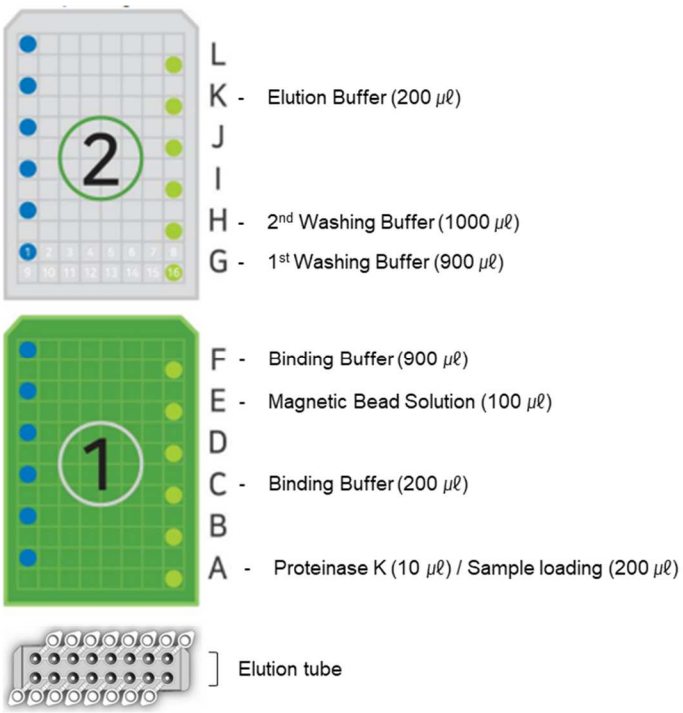


Figure 1. Position of reagents, tubes, and starting material in Cartridges/Elution Tube Rack of *ExiPrep*™ Plus Blood Genomic DNA Kit (K-4211).

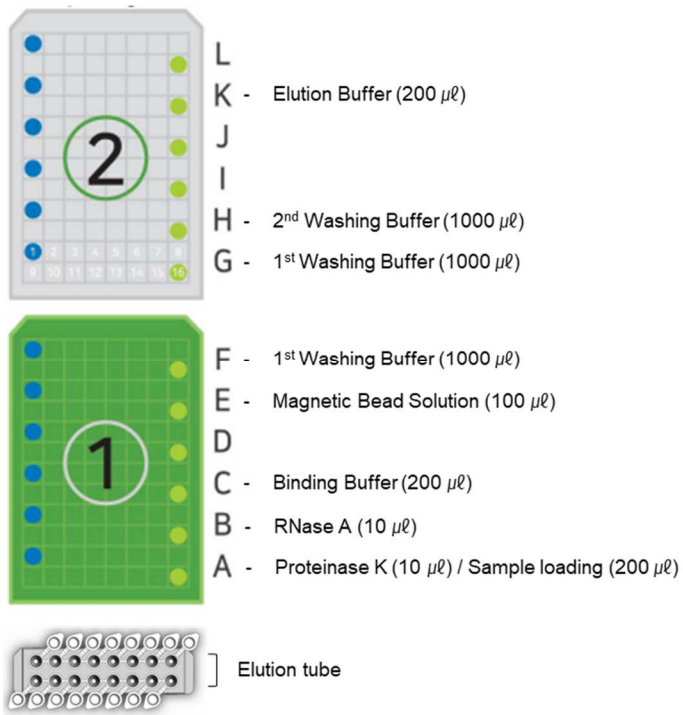


Figure 2. Position of reagents, tubes, and starting material in Cartridges/Elution Tube Rack of *ExiPrep*™ Plus Bacteria Genomic DNA Kit (K-4214).

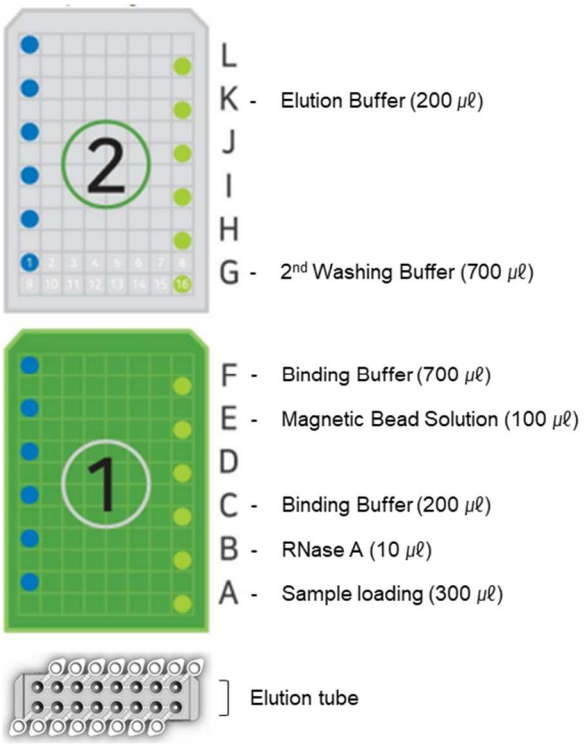


Figure 3. Position of reagents, tubes, and starting material in Cartridges/Elution Tube Rack of *ExiPrep*™ Plus Plant Genomic DNA Kit (K-4215).

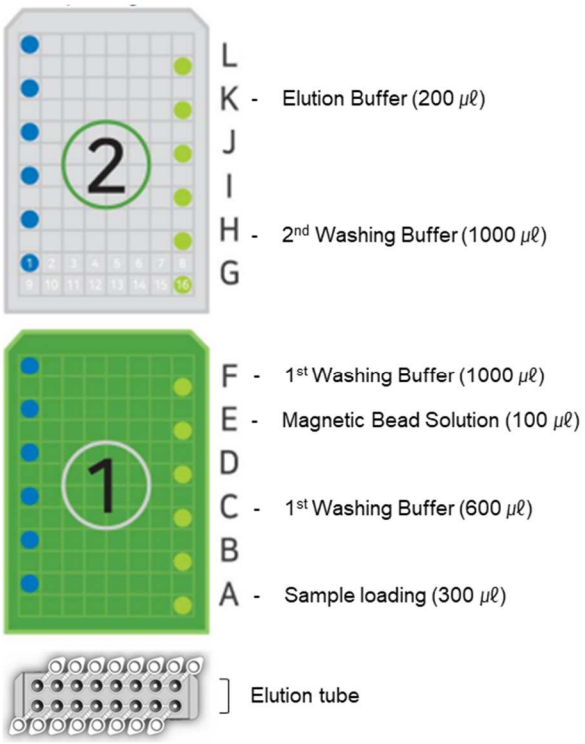


Figure 4. Position of reagents, tubes, and starting material in Cartridges/Elution Tube Rack of *ExiPrep*™ Plus Seed Genomic DNA Kit (K-4217).

Experimental Procedures

Preparing Sample from Whole Blood and Buffy Coat

1. If the sample volume is less than 200 µl, adjust the total volume to 200 µl by adding PBS buffer (not provided) to achieve maximum lysis efficiency.

2. Proceed immediately to “Loading the Kit to the Instrument” on page 12.

Preparing Sample from Cultured Cells

1. Harvest cells according to step 1-A or 1-B.

1-A. Suspension cell culture:

Harvest cultured cells (< 1 x 10^6 cells) by centrifugation at 3,000 rpm for 5 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet. Wash the pellet with sterile PBS buffer (not provided) and re-centrifuge to pellet cells. Go to step 2.

1-B. Monolayer cell culture:

There are two different methods to collect cells grown in monolayer culture.

a. Direct cell harvesting on the culture dishes:
   Completely discard the cell culture medium and go to step 2.
   * **Note**: You should completely remove the cell culture medium because it may inhibit the DNA extraction.

b. Cell harvesting with trypsin:
   Remove the cell culture medium and wash the cell monolayer with PBS buffer. Add 0.1-0.25% trypsin to the washed cell monolayer to detach the cells. After the cells detach, add cell culture medium. Transfer the cells to a clean tube and centrifuge at 3,000 rpm for 5 minutes. Discard the supernatant carefully and go to step 2.

2. Resuspend the cell pellet or monolayer from step 1 in 200 µl of PBS buffer.

3. Proceed immediately to “Loading the Kit to the Instrument” on page 12.

Preparing Sample from Bacterial Cells (Gram-Negative Bacteria)

1. Harvest up to 1 x 10^9 bacterial cells by centrifugation at 8,000 rpm for 5 minutes to pellet cells. Discard the supernatant carefully with a pipette.

2. Resuspend the cell pellet from step 1 in 200 µl of Resuspension Buffer.

3. Proceed immediately to “Loading the Kit to the Instrument” on page 12.

Preparing Sample from Bacterial Cells (Gram-Positive Bacteria)

1. Harvest up to 1 x 10^9 bacterial cells by centrifugation at 8,000 rpm for 5 minutes to pellet cells. Discard the supernatant carefully with a pipette.

2. Resuspend the cell pellet from step 1 in 200 µl of 1X TE buffer (not provided) by vortexing or pipetting.

3. Add 20 µl of lysozyme (50 mg/ml, not provided) and incubate at 37°C for at least 1 hour to form spheroplasts.

   * **Note:** If you proceed with yeast, add zymolyase with 0.1% of β-mercaptoethanol. The final concentration of zymolyase is 50 U per 1 x 10^6 cells. Incubate at 30°C for 30 minutes.

4. Centrifuge at 13,000 rpm for 5 minutes and discard the supernatant carefully with a pipette.

5. Resuspend the cell pellet in 200 µl of Resuspension Buffer.

6. Proceed immediately to “Loading the Kit to the Instrument” on page 12.

Preparing Sample from Plant Tissue

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

Before beginning, completely dissolve 20 mg of Proteinase K powder in 1 ml of DNase- and RNase-free water.

1. Grind (or homogenize) ≤ 100 mg (fresh) or 10-20 mg (dry) of plant sample with a mortar and pestle (or homogenizer) to a fine powder in liquid nitrogen and place them into a clean 1.5 ml tube.
   * **Note:** If the sample is not ground completely, it will result in significantly reduced DNA yields. Plant tissue or seeds can be disrupted with mechanical bead-based methods instead of liquid nitrogen.
2. Add 300 µl of Plant Lysis Buffer and 20 µl of Proteinase K (20 mg/ml) to the sample.
   * **Note:** The sample should be completely immersed in the buffer.
3. Incubate at 60°C for at least 30-60 minutes with a shaking water bath.
4. Centrifuge at 13,000 rpm for 5 minutes to remove any precipitates.
5. Transfer the cleared lysate to a new 1.5 ml tube (not provided).
6. Proceed immediately to “Loading the Kit to the Instrument” on page 12.

Preparing Sample from Plant (GMO) Seeds

Before beginning, completely dissolve 20 mg of Proteinase K powder in 1 ml of nuclease-free water.

Before beginning, completely dissolve 24 mg of RNase A powder in 600 µl of nuclease-free water.

1. Grind (or homogenize) ≤ 50 mg (fresh) or 10-20 mg (dry) of plant sample with a mortar and pestle (or homogenizer) to a fine powder in liquid nitrogen and place them into a clean 1.5 ml tube.

*** Note:** If the sample is not ground completely, it will result in significantly reduced DNA yields. Plant tissue or seeds can be disrupted with mechanical bead-based methods instead of liquid nitrogen.

2. Add 270 µl of Seed Lysis Buffer, 20 µl of Proteinase K (20 mg/ml), and 10 µl of RNase A (40 mg/ml) to the sample.

*** Note:** The sample should be completely immersed in the buffer.

3. Incubate at 60°C for 10 minutes with a shaking water bath.

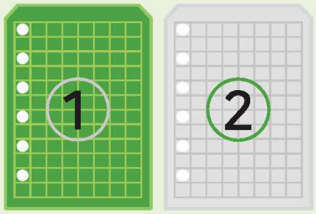
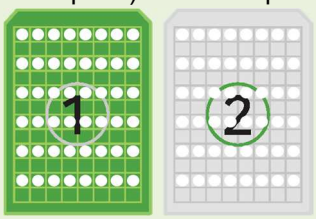
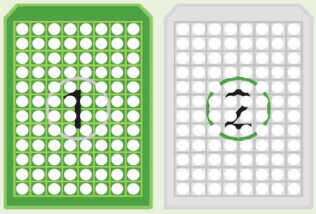
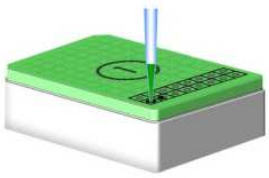
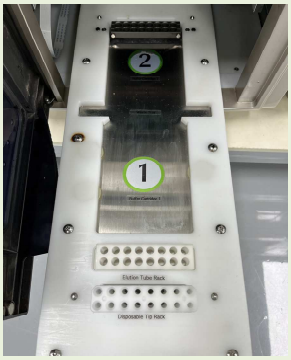
4. Add 90 µl of Precipitation Buffer into the sample and incubate tubes on ice for 5 min.

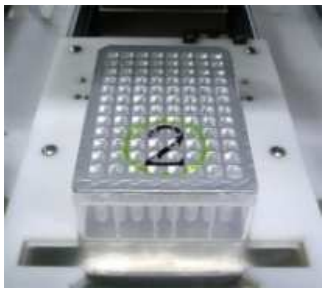
5. Centrifuge at 13,000 rpm for 5 minutes to remove any precipitates.

6. Transfer the cleared lysate to a new 1.5 ml tube (not provided).

7. Proceed immediately to “Loading the Kit to the Instrument” on page 12.

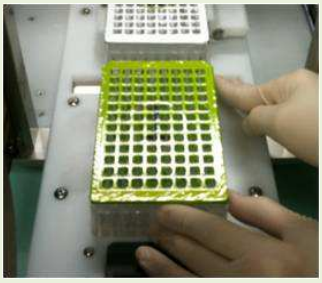
Loading the Kit to the Instrument

Steps	Procedure Details
Example 1) For 1 sample  Example 2) For 8 samples  Example 3) For 16 samples 	1. Punch holes in the sealing films of Buffer Cartridge ① and ② using 6-Hole Punch (*ExiPrep*™ 16 Plus's accessory) according to the number of samples. *** Note:** Before punching the hole, agitate the Buffer Cartridge gently to settle the beads and buffer.
	2. Load 200-300 µl of sample prepared in pp. 8-11 into 'Sample loading well' of Cartridge ①. *** Note:** Be careful not to contaminate any other wells.
	3. Open the door of *ExiPrep*™ 16 Plus and pull out the baseplate completely.

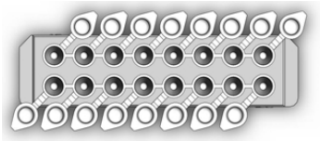


4. Place the Buffer Cartridge ② onto the proper position of the baseplate.

*** Note:** Please check the punched holes of the Buffer Cartridge ②.

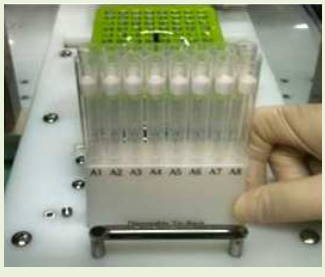


5. Place the Buffer Cartridge ① onto the proper position of the baseplate.



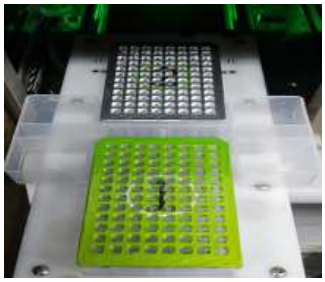
6. Place the Elution Tube Rack including Elution Tubes onto the proper position of the baseplate. The Elution Tube Rack is slotted so it can only be placed in the correct orientation.

*** Note:** Make sure the direction of the Elution Tube caps laid out as on the left when inserting into the Elution Tube Rack.



7. Place the Disposable Filter Tip Rack onto the proper position of the base plate.

*** Note:** Tips should be placed in the corresponding positions with the punched holes of the Cartridges.



8. Place the Waste tray between Buffer Cartridge ① and ②.

9. Finally, confirm holes in the cartridges and position of samples and tips. Push the baseplate completely until you hear the click sound, then close the door.

10. Turn on the *ExiPrep*™ 16 Plus.

11. In the MENU screen, press 'Start' button to select a proper protocol.

12. The PREP SETUP screen appears as shown in the left, press protocol number according to the protocol number list (pp. 17-18). Confirm following information displayed on the screen, and then press the 'Enter' button.

13. Select the desired elution volume from the touch screen.

14. Press the 'ok' button to move to the next step.

15. Ensure that everything is correctly installed following the CHECK LIST, then choose "ok".

16. In the Running Mode screen, ensure that the protocol name appears on the screen.

17. Press the "RUN" button to initiate DNA extraction.

18. "Work Completion" screen appears when the protocol is completed. Open the door and collect final DNA from the Elution Tubes.

19. Remove all components used in the experiment, and choose 1, 2, or ok.

*** Note:** If you want to quit and press the 'ok' button, the UV lamp will be turned on automatically.

Troubleshooting

Problem	Comments
Low DNA yield or purity	• **You may have used too much (or too little) starting material.** DNA yield is dependent on the sample type and amount of starting sample. Appropriate amount of starting sample should be used for efficient extraction of genomic DNA. For mor information, refer to “Specifications” on page 2.
	• **The lysis may have been incomplete.** Centrifuge completely to obtain clear lysate.
	• **Incomplete suspension of the magnetic nanobeads may decrease the DNA yield or purity.** You should agitate the Buffer Cartridge ① before use.
Co-eluted magnetic nanobeads	Sometimes magnetic nanobeads are eluted with your genomic DNA. Magnetic nanobeads in the eluate will not affect the performance of the genomic DNA in downstream applications. Furthermore, magnetic nanobeads cannot bind genomic DNA in elution buffer, though it may affect readings on a spectrophotometer. Magnetic nanobeads that are carried over can be easily separated by centrifugation at 13,000 rpm for 1 minute.

Appendix A

Genomic DNA Extraction from Yeast

1. Harvest cultured cells (< 1 x 10^9 cells) by centrifugation to pellet cells. Discard the supernatant carefully with a pipette.

2. Resuspend the cell pellet in 1 ml of PBS buffer (not provided) by vortexing or pipetting.

3. Centrifuge at 13,000 rpm at room temperature for 5 minutes and discard the supernatant with a pipette.

4. Resuspend the cell pellet in 200 µl of 1X TE buffer (not provided) by vortexing or pipetting.

5. Add 20 µl of lysozyme (50 mg/ml, not provided) and incubate at 37°C for at least 1 hour.
   * **Note:** If you proceed with yeast, add zymolyase with 0.1% of β-mercaptoethanol. The final concentration of zymolyase is 50 U per 1 x 10^6 cells. Incubate at 30°C for 30 minutes.

6. Incubate at 30°C for 30 minutes.

7. Centrifuge at 13,000 rpm for 5 minutes and discard the supernatant carefully with a pipette.

8. Resuspend the cell pellet in 200 µl of Resuspension Buffer.

9. Proceed immediately to “Loading the Kit to the Instrument” on page 12.

Appendix B

List of Protocol Numbers

Protocol Number	Target	Sample Source
No. 101	Genomic DNA	Whole blood
No. 102	Genomic DNA	Animal tissue
No. 103	Genomic DNA	FFPE tissue
No. 104	Genomic DNA	Plant tissue
No. 105	Genomic DNA	Plant seed
No. 106	Genomic DNA	Rice
No. 107	Genomic DNA	Cultured cell
No. 108	Genomic DNA	Gram (+) bacteria cell
No. 109	Genomic DNA	Gram (-) bacteria cell
No. 110	Genomic DNA	Yeast
No. 111	Genomic DNA	Fungi
No. 114	Genomic DNA	Buffy coat
No. 115	Genomic DNA	Sputum
No. 116	Genomic DNA	BAL
No. 117	Genomic DNA	Saliva
No. 118	Genomic DNA	Swab
No. 119	Genomic DNA	Urine
No. 110	Genomic DNA	Stool
No. 123	Genomic DNA	CSF
No. 124	Genomic DNA	EPS
No. 125	Genomic DNA	Respiratory sample
No. 126	Genomic DNA	Amniotic fluid
No. 127	Genomic DNA	Forensic sample
No. 128	Genomic DNA	Bone marrow

No. 129	Genomic DNA	Bone
No. 130	Genomic DNA	Dried blood spot
No. 131	Genomic DNA	Soil
No. 132	Genomic DNA	Hair
No. 133	Genomic DNA	Cell supernatant

Ordering Information

Description	Cat. No
ExiPrep™ Plus Blood Genomic DNA Extraction Kit	K-4211
ExiPrep™ Plus Bacteria Genomic DNA Extraction Kit	K-4214
ExiPrep™ Plus Plant Genomic DNA Extraction Kit	K-4215
ExiPrep™ Plus Seed Genomic DNA Extraction Kit	K-4217

Related Products

Description	Cat. No
ExiPrep™16 Plus	A-5030
ExiProgen™	A-5041
Proteinase K Powder	KB-0111
RNase A Powder	KB-0101
Phosphate Buffered Saline (PBS)	C-9024

Explanation of Symbols

LOT Batch Code	Biological Risks	REF Catalog Number	Caution
Consult Instructions For Use	Contains Sufficient for <n> tests	Do not Re-use	Manufacturer
RUO Research Use Only	Temperature Limitation	Use-by Date	

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