USER GUIDE

ExiPrep™ Tissue Genomic DNA Kit

Cat. No. K-3225

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ExiPrep™ Tissue Genomic DNA Kit

Kit for the extraction of genomic DNA from animal tissue

User Guide

K-3225

Σ 96

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Please read all the information in booklet before using the unit

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Intended Use

ExiPrep™ Tissue 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	Amount
Buffer Cartridge ①	6 ea
Buffer Cartridge ②	6 ea
Tissue Lysis Buffer	25 ml x 1 ea
Proteinase K powder, lyophilized	20 mg x 2 ea
Reaction Tube	1 pack (96 ea/pack)
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.
This product also contains lyophilized enzymes (Proteinase K and RNase A), which are pre-loaded into Buffer Cartridges (RNase A) and 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
Animal tissue (Bovine muscle)	10-40 mg	5-10 μg	A_{260}/A_{280} > 1.8
Animal tissue (Rat tail tip)	0.5 cm	5-10 μg	
Cultured cells (HeLa cells)	< 1 x 10^6 cells	5-15 μg	

*** 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™ Tissue Genomic DNA Kits are designed for extraction of highly purified total DNA from animal tissues. *ExiPrep*™ Genomic DNA Kits provide total solution for accurate and rapid total 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 (Cat. No. A-5010). *ExiPrep*™16 is designed for rapid extraction of nucleic acids delivering up to 16 extracted samples automatically.

Protocol of *ExiPrep*™ Tissue Genomic DNA Kit requires a sample disruption step by using Proteinase K in the supplied Tissue Lysis 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, southern blotting, and SNP genotyping.

Principle

ExiPrep™ Tissue Genomic DNA Kit is designed for extraction of genomic DNA. This kit employs 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 such as animal tissues, cultured cells, or FFPE tissues.
- 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

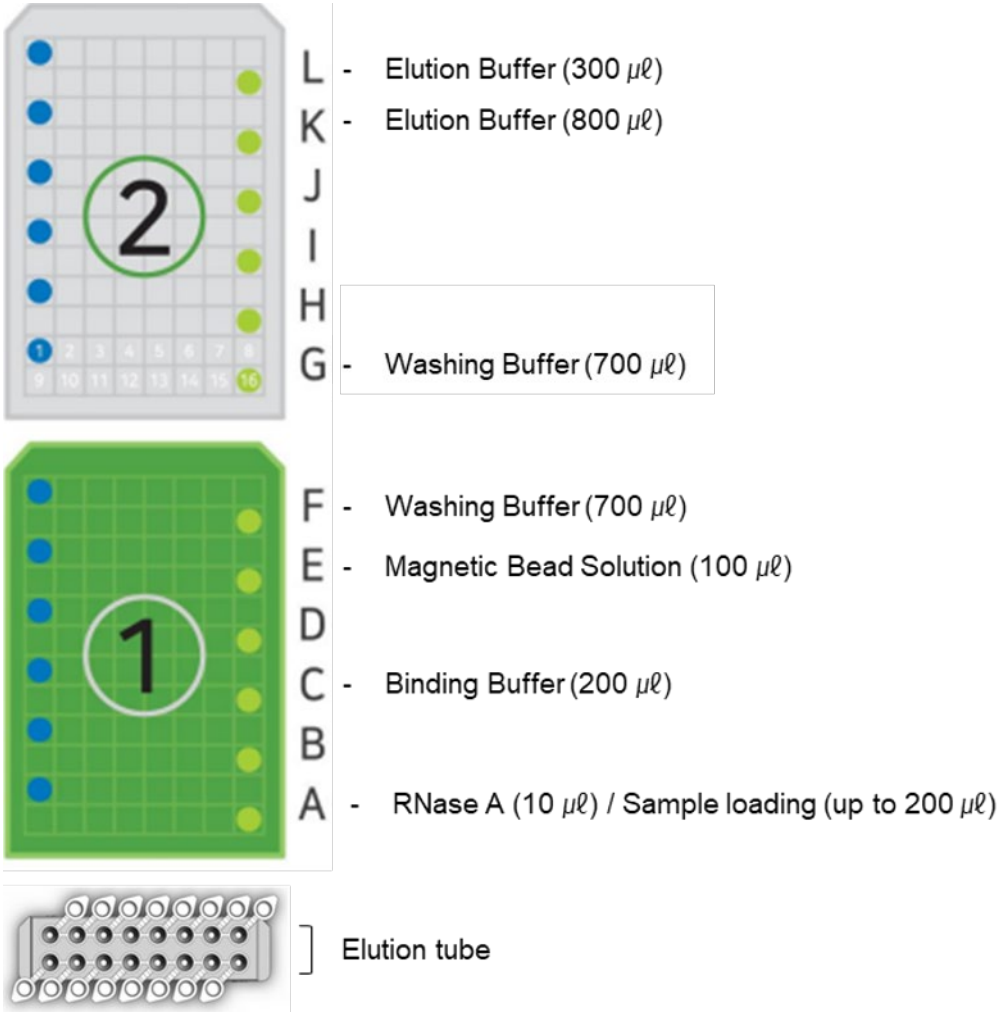


Figure 1. Position of reagents, tubes, and starting material in Cartridges/Elution Tube Rack of *ExiPrep*™ Tissue Genomic DNA Kit (K-3225).

Experimental Procedures

Preparing Sample from Animal Tissues

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

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

Disrupt tissues according to step 1, 2, or 3.

1. Tissue samples can be disrupted before Proteinase K digestion by pestle and mortar with or without liquid nitrogen.

1) Grind 10-40 mg of animal tissue sample in liquid nitrogen† to a fine powder with a mortar and pestle and place it into a 1.5 ml tube.

*** Note:** Do not allow the sample to thaw.

† After grinding, liquid nitrogen should be evaporated.

2) Add 200 µl of Tissue Lysis Buffer† and 20 µl of Proteinase K (20 mg/ml) to the sample.

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

† Storage of Tissue Lysis Buffer at lower temperatures may cause precipitation. If precipitated, heat the buffer at 60°C to re-dissolve.

3) Incubate at 60°C for at least 2 hours 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 10.

2. Tissue samples can be disrupted by overnight digestion with Proteinase K.

1) Grind 10-40 mg of animal tissue sample and place it into a 1.5 ml tube.

2) Add 200 µl of Tissue 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 overnight with a shaking water bath.

4) Proceed immediately to “Loading the Kit to the Instrument” on page 10.

3. Tissue samples can be disrupted by BIONEER’s Tissue Homogenization Grinder (Cat. No. KA-7034) and Tissue Homogenization Sticks (Cat. No. KA-7031) before Proteinase K digestion.

1) Cut up 10-40 mg of animal tissue sample and place it into a *AccuPrep*® Filtering Columns (not provided, Cat. No. KA-1160).

2) Add 200-400 µl of Tissue Lysis Buffer to the sample.

3) Homogenize the sample with a Tissue Homogenization Grinder or Tissue Homogenization Sticks. Make sure to completely disrupt the sample.

4) Centrifuge at 13,000 rpm for 2 minutes.

5) Take the flow-through in the Collection tube to a new 1.5 ml tube.

6) Add 20 µl of Proteinase K (20 mg/ml) to the lysate.

7) Proceed immediately to “Loading the Kit to the Instrument” on page 10.

Preparing Sample from Cultured Cells

Storage of resuspension buffer at lower temperatures may cause precipitation. If precipitated, heat at 60°C until the precipitate is completely dissolved.

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.

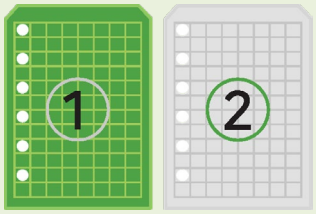
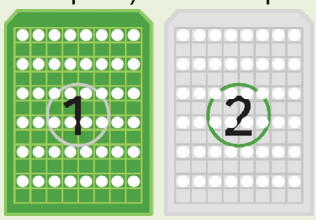
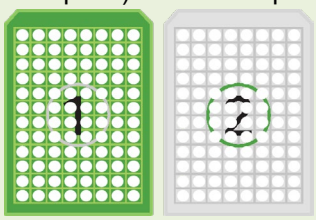
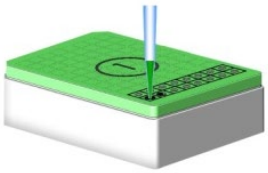
a. Direct cell harvesting on the culture dishes:
   Completely discard the cell culture medium and go to step 2.
   * **Note**: You should completely remove the cell culture medium because it may inhibit the DNA extraction.

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.

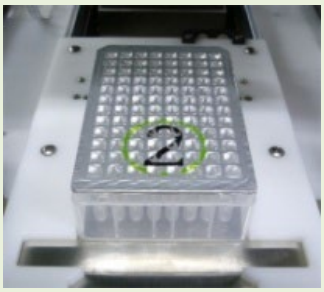
2. Resuspend the cell pellet or monolayer from step 1 in 200 µl of Tissue Lysis Buffer.

3. Proceed immediately to “Loading the Kit to the Instrument” on page 10.

Loading the Kit to the Instrument

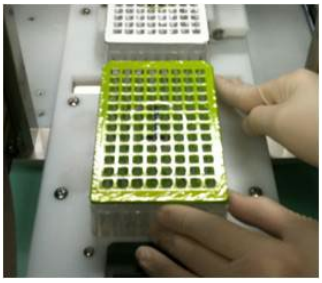
Steps	Procedure Details
Example 1) For 1 sample  Example 2) For 8 samples  Example 3) For 16 samples  	1. Punch holes in the sealing films of Buffer Cartridge ① and ② using 6-Hole Punch (*ExiPrep*™ 16's accessory) according to the number of samples. * **Note:** Before punching the hole, agitate the Buffer Cartridge gently to settle the beads and buffer.
	2. Load 200 µl of samples prepared in pp. 7-9 into 'Sample loading well' of Cartridge ①. * **Note:** Be careful not to contaminate any other wells.
 	3. Open the door of *ExiPrep*™ 16 and pull out the baseplate completely.

4. Place the Reaction Tube Rack including Reaction Tubes onto the proper position of the baseplate.

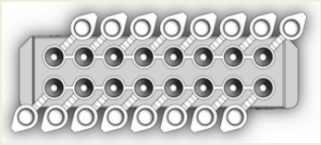


5. Place the Buffer Cartridge ② onto the proper position of the baseplate.

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

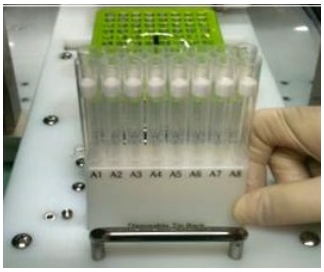


6. Place the Buffer Cartridge ① onto the proper position of the baseplate.



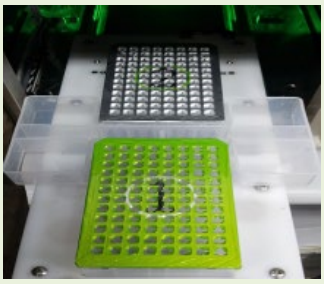
7. Place the Elution Tube Rack including Elution Tubes onto the proper position of the baseplate. The Elution Tube Rack is slotted so it can only be placed in the correct orientation.

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



8. 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.



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

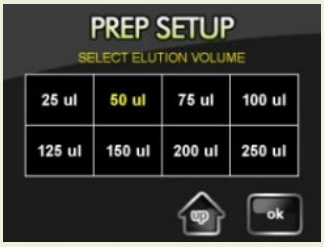
10. Finally, confirm holes in the cartridges and position of samples and tips. Push the baseplate completely until you hear the click sound, then close the door.

11. Turn on the *ExiPrep*™ 16.

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

13. The PREP SETUP screen appears as shown in the left, and a screen to select the protocol number for each kit appears. Press **'102' or '103'** according to sample type. Confirm following information displayed on the screen, and then press the 'Enter' button.

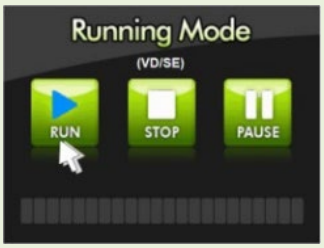
Protocol. No.: 102	**Protocol. No.: 103**
Prep Type: Genomic DNA	**Prep Type: Genomic DNA**
Sample SRC: Animal tissue	**Sample SRC: FFPE tissue**



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

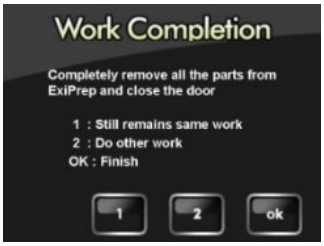
15. Press the 'ok' button to move to the next step.

16. Ensure that everything is correctly installed following the CHECK LIST, then choose “ok”.



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

18. Press the “RUN” button to initiate DNA extraction.



19. “Work Completion” screen appears when the protocol is completed. Open the door and collect final DNA from the Elution Tubes.

20. 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 FFPE Tissue

1. Place the 1 piece of sectioned FFPE tissue into a 1.5 ml tube.

2. Add 1 ml of xylene to the sample and vortex for 30 seconds.

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

4. Add 1 ml of absolute ethanol and vortex for 30 seconds.

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

6. Repeat steps 4-5 once more.

7. Dry the sample at 60°C in a heating block or oven to completely evaporate the residual ethanol.

8. Go to step B of the “Preparing Sample from Animal Tissues” on page 7.

Ordering Information

Description	Cat. No
ExiPrep™ Tissue Genomic DNA Kit	K-3225

Related Products

Description	Cat. No
ExiPrep™16	A-5010
ExiProgen™	A-5041
Proteinase K Powder	KB-0111

Explanation of Symbols

EC REP Authorized Representative in the European Community	LOT Batch Code	Biological Risks	REF Catalog Number
Caution	Consult Instructions For Use	Contains Sufficient for <n> tests	Do not Re-use
IVD *In vitro* Diagnostic Medical Device	Manufacturer	RUO Research Use Only	Temperature Limitation
Use-by Date			

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