ISO 9001
Certified

Ver. 1.0

User's Guide

Global Genomics Partner

ExiPrep™ Tissue total RNA Kit

(K-3325)

BIONEER
bioneer corporation

Safety Warnings and Precautions

ExiPrep™ Tissue total RNA 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™ Tissue total RNA Kit
Buffer Cartridge ①	6 ea
Buffer Cartridge ②	6 ea
Disposable Tip	96 ea
Reaction Tube (0.5ml)	96 ea
Elution Tube (8-strip)	12 ea
User's Guide	1 ea
Tissue lysis buffer ①	1 ea
Tissue lysis buffer ②	1 ea

II. Storage

ExiPrep™ Tissue total RNA Kit provides 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™ Tissue total RNA Kit provides optimized tissue lysis buffer ① and ②. The Tissue lysis buffers were must kept under 4 ℃ and dark condition. The tissue lysis buffer ① and ② were poisonous contact with skin will cause burns. After contact with skin, wash immediately with plenty of water and detergent. Use gloves and eye protection when working with tissue lysis buffer ① and ②

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

III. Introduction

The *ExiPrep*™ Tissue total RNA Kit is suitable to extract of total RNA from various type of animal tissue samples e.g. liver, lung, heart, kidney, brain, eye and tail tip. Some kinds of samples need special pre-treatment step together with provided special buffers.

Ribonucleases (RNase) are very stable and extremely active enzymes that do not required co-factors. RNases are quite difficult to inactivate and small amounts are enough to destroy RNA. To minimize RNase contamination the following guidelines should be taken when working with RNA.

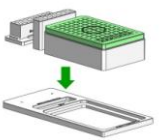
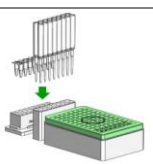
- Always wear latex or vinyl gloves and change gloves often. RNases arise readily from bacteria and molds present in dust and on skin and clothing.
- Use sterile, disposable plasticware to prevent cross-contamination with RNases from shared equipment.
- Non-disposable plasticware or glassware can be washed with detergent, rinsed several times with sterile distilled water, followed by thorough rinses with 0.1 N NaOH, 1mM EDTA and, finally, RNase-free sterile distilled water.
- Alternatively, glassware can be washed with detergent, well rinsed and baked in a dry oven at 240℃ for 4 or more hours. Please note that autoclaving will not completely inactivate all RNases. Glassware can be treated with DEPC (diethyl pyrocarbonate) by filling glassware with 0.1% DEPC, left to stand 12 hrs at 37℃ or more hours and then autoclaved to eliminate the DEPC.
- Electrophoresis tanks are cleaned with detergent, rinse several times with RNase-free water, rinsed with ethanol and allowed to dry.

IV. Starting Volume

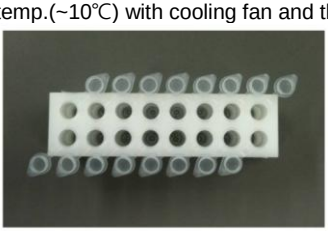
The amounts of starting volume and elution volumes are described in below.

Sample type	Starting Volume	Elution Volume
Liver	20mg	100ul
Lung	20mg	100ul
Kidney	20mg	100ul
Spleen	20mg	100ul
Brain	20mg	100ul
Tail tip	0.5cm	100ul
Eye	1 ea	100ul

V. Set-up the Consumables and the Racks

	1. Make holes with the hole-punch tool of the Buffer cartridge ① and ② to correspond with the sample numbers. ※ Before punching the hole, shake the Buffer cartridge ① gently.
	2. Place the Buffer Cartridge ①, Elution tube rack, Reaction tube rack and Disposable tip rack on the setup tray.
	3. Load the Elution tubes, Reaction tubes and Disposable filter tips 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.



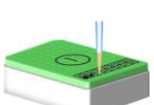
Elution tube & Elution tube rack for the *ExiPrep*™ 16 Plus

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

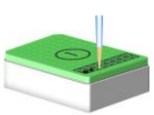
VI. Pre-treatment steps for tissue disruption

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

A. Tissue samples can be disrupted by pestle & mortar with liquid nitrogen.

	a. Add liquid nitrogen into the mortar to cooling the mortar and pestle. b. Cut up to 10-20mg of animal tissue and transfer into the mortar. c. Make a fine powder with liquid nitrogen.
	d. Transfer the powdered tissue into 1.5ml test tube. (not provided) e. Add 400 ul of Tissue lysis buffer ① and mix by vortexing for 15 sec.
	f. Add 200ul of Tissue lysis buffer ② and mix by vortexing for 15 sec. g. Incubate the tube on ice for 5 min.
	h. Centrifuge the tube at 12,000 rpm, 4℃ for 15 min. to remove the incompletely lysed tissue and cell debris. i. And take the supernatant only without contact the intermediate cell debris layer.
	j. Load the supernatant into the Sample loading well of the Buffer cartridge ①.

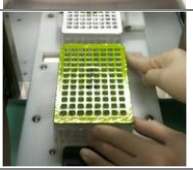
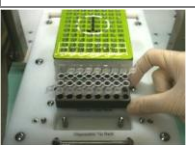
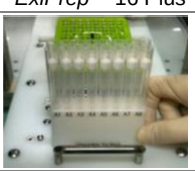
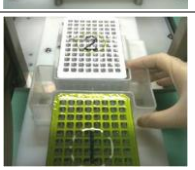
B. Tissue samples can be disrupted by pestle & mortar with Tissue lysis buffer ①.

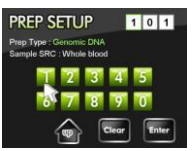
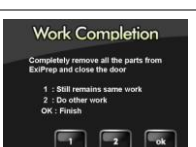
	a. Cut up to 10-20mg of animal tissue and transfer into the mortar. b. Add 500ul of Tissue lysis buffer ①. c. Grind the tissue sample, gently.
	d. Transfer the lysed tissue lysate into 1.5ml test tube. (not provided) e. Add 200ul of Tissue lysis buffer ② and mix by vortexing for 15 sec. f. Incubate the tube on ice for 5 min.
	g. Centrifuge the tube at 12,000 rpm, 4℃ for 15 min. to remove the incompletely lysed tissue and cell debris. h. And take the supernatant only without contact the intermediate cell debris layer.
	i. Load the supernatant into the Sample loading well of the Buffer cartridge ①.

C. Tissue samples can be disrupted by mechanical disrupter with Tissue lysis buffer ①.

	a. Cut up to 10-20mg of animal tissue and transfer into the 1.5ml test tube. b. Add 500ul of Tissue lysis buffer ①. c. Load the appropriate volume of beads (glass or iron) into the tube.
	d. Homogenization with mechanical disrupter e.g. bead mixer, vortex mixer. e. Add 200ul of Tissue lysis buffer ② and mix by vortexing for 15 sec. f. Incubate the tube on ice for 5 min.
	g. Centrifuge the tube at 12,000 rpm, 4℃ for 15 min. to remove the incompletely lysed tissue and cell debris.
	h. And take the supernatant only without contact the intermediate cell debris layer. i. Go to Step 1 of the 'Total RNA Extraction from Animal tissue'. (Page 9)

VII. Total RNA Extraction from Animal tissue

<table>
<tr><td>  </td><td colspan="2">1. Place the Reaction tube Rack onto the proper position of the base plate.
(ExiPrep™ 16 instrument only)</td></tr>
<tr><td>  </td><td colspan="2">2. Place the Buffer Cartridge ① and ② onto the proper position of the base plate.
Please check the punched holes of the Buffer Cartridge ① and ②.</td></tr>
<tr><td> 
ExiPrep™ 16 Plus</td><td> 
ExiPrep™ 16 Pro</td><td>3. Place the Elution tube rack onto the proper position of the base plate.</td></tr>
<tr><td>  </td><td colspan="2">4. Place the Disposable filter tip rack onto the proper position of the base plate.</td></tr>
<tr><td>  </td><td colspan="2">5. Place the Waste tray onto the proper position of the base plate between Buffer cartridge ① and Buffer cartridge ②.
6. Push the base plate back into the instrument and close the door.</td></tr>
</table>

	7. Turn on the *ExiPrep*™ 16 Plus/ Pro. 8. Press the 'Start' button to access the PREP SETUP menu.
	9. Insert a protocol number according to the protocol number list(Page 15) about nucleic acid types and sample sources. 10. Press the 'Enter' button to move to the next step.
	11. Select a elution volume from the touch screen. 12. Press the 'ok' button to move to the next step.
	13. Verify the loaded every racks and buffer cartridges in the correct position on the base plate according to the 'CHECK LIST' like as followings.
	14. 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. 15. Press the 'Run' button to start an extraction run.
	16. After the completion of the Instrument's operation, take the Elution tube from base plate first. 17. Remove the buffer cartridges, each racks and Waste tray from the base plate and close the door.

VIII. Troubleshooting

1. Low yield
- Incomplete homogenization or lysis of samples.

2. Low A_{260}/A_{280} ratio (<1.6)
- The aqueous phase was contaminated with the phenol phase.

3. RNA degradation
- Tissues were not immediately processed or frozen after removing from animal.
- Samples used for isolation, or the isolated RNA were not stored long-term at -70℃.

4. DNA contamination
- Sample was homogenized in too small a reagent volume.
- Sample contained organic solvents (e.g., ethanol, DMSO), strong buffers or alkaline solution.

IX. Protocol Number List for total RNA extraction

No.	Sample source
201	Whole blood
202	Animal tissue
203	FFPE tissue
204	Plant tissue
205	Plant seed
206	Rice
207	Cultured cell
208	Gram (+) bacteria
209	Gram (-) bacteria
210	Yeast
211	Fungi
214	Buffy coat
215	Sputum
216	BAL
217	Saliva
218	Swab
219	Urine
220	Stool
223	CSF
224	EPS
225	Respiratory sample
226	Amniotic fluid
227	Forensic sample
228	Bone marrow
229	Bone
230	Dried blood spot
231	Soil
232	Hair
233	Cell supernatant

X. Ordering Information

Product	Size	Cat. No.
ExiPrep™ 16 Plus	1 ea	A-5030
ExiPrep™ 16 Pro	1 ea	A-5040
ExiPrep™ Blood Genomic RKit	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™ Tissue Total RNA Kit	96 prep.	K-3325
ExiPrep™ Cell Total RNA Kit	96 prep.	K-3335
ExiPrep™ Viral DNA/ RNA Kit	96 prep.	K-3535