ExoLutE® URINE
Exosome Isolation Kit

USER GUIDE

Catalog Number EX-02
Revision B

BEFORE FIRST USE

"PLEASE READ CAREFULLY AND FOLLOW ALL THE INSTRCUTION"

EVEN IF YOU FEEL YOU ARE FAMILIAR with the products.

Store at 4˚C upon arrival.

RESEARCH USE ONLY

Rosetta Exosome®

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TRADEMARKS

BEFORE FIRST USE

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I

Product information

- Background
- Reagents provided and storage condition
- Consumables and laboratory equipment to be supplied by user
- Workflow

Background

Extracellular vesicles, also known as exosomes and microvesicles, are nano-vesicles (30-1,000 nm in diameters) produced by most living cells on the earth and composed of a variety of cellular components including proteins, lipids, and nucleic acids that are originated from their parental cells. These are found abundantly in the body fluids such as the blood, urine, saliva, cerebrospinal fluid, and breast milk.

Currently, extracellular vesicles are recognized as important biological mediators for intercellular communication in a variety of physiological events, where extracellular vesicles shuttle their cargo between cells. Over the past decade, molecular composition in extracellular vesicles has been proved to be associated with certain diseases and treatment responses, indicating that extracellular vesicles hold a great promise for diagnostic and prognostic tools for various diseases, as well as for therapeutic targets. Despite their importance, extracellular vesicle isolation is still considered as a major challenge, since both conventional methods with one-dimensional separation and tools in the field have not been satisfactory for excluding contaminants in the final products.

The science team in Rosetta Exosome Inc. has developed a novel multi-dimensional extracellular vesicle isolation workflow. This workflow comprises unique extracellular vesicle isolation technologies combined with precisely tuned spin-based size-exclusion chromatography. Using the workflow, extracellular vesicles in various biological fluids can be rapidly and successfully purified in intact form with superior purity as compared with other methods. Moreover, each line of ExoLutE® Exosome Isolation Kit is specifically designed for corresponding biological fluids such as mammalian cell-conditioned media, the cerebrospinal fluids, urine, plasma, and serum. The ExoLutE® Exosome Isolation Kit is composed of all the necessary reagents and materials which permit a simple and reliable method to produce **HIGHLY PURE**, **CONCENTRATED**, and **INTACT extracellular vesicles** for basic researches, biomarker discoveries, and clinical applications.

IMPORTANT NOTE: All currently available 'ExoLutE® Exosome Isolation Kits' are designed for the purification of exosomes and microvesicles, collectively as extracellular vesicles!

Reagents provided and storage condition

Each reaction in this kit (10 reactions) is designed for purifying extracellular vesicles from 8 mL of the biological fluid such as urine. All reagents included in ExoLutE® Exosome Isolation Kit are sterilized by 0.22 μm pore-sized filters (but without preservatives). Take care to keep the reagent sterile after use.

Table 1 ExoLutE® Urine Exosome Isolation Kit: 10 reactions

Component [1,2]	Amount	Part number	Storage
Sol U	30 mL	EX02L01	4°C
Sol A	6 mL	EX01L01	4°C
Sol B	20 mL	EX01L02	4°C
Sol C	50 mL	EX01L03	4°C
Sol R	6 mL	EX01L04	4°C
Spin-SEC column	10 EA	EX01C03	4°C
Column bottom cap	10 EA	EX01S03	RT[3]

[1] Store the components at the designated condition for up to 12 months.
[2] The stability of the kit will not be affected at room temperature during shipping.
[3] Room temperature.

Consumables and laboratory equipment to be supplied by the user

Unless otherwise, all materials are available through other major laboratory suppliers.

Consumables:

- 15 mL conical centrifuge tubes.
- 1.5 mL and 2.0 mL microcentrifuge tubes.
- 100 kDa molecular weight cut-off (MWCO) filter units (*e.g.* Amicon centrifugal filter devices or equivalents).
- 0.45 μm pore-sized filters (or 0.8 μm pore-sized filters).
- Common culture media such as DMEM and RPMI1640.

Equipment:

- Vortex mixer.
- Rocker or an equivalent instrument for 15 mL falcon tubes and microcentrifuge tubes.
- Bench-top microcentrifuges for 1.5 mL or 2.0 mL microcentrifuge tubes.
- Laboratory-scale centrifuge (**swinging-bucket rotor**, 3,000 *xg* or higher) and appropriate **adaptors** for 50 ml and 15 mL conical centrifuge tubes.
- Personal protection equipments (lab coat, gloves, and goggles).

II

ExoLutE® URINE
Exosome Isolation Kit Protocol

Rosetta Exosome®

- Procedure guides
- Pre-clearing
- (Optional) Concentrating materials
- Extracellular vesicle enrichment
- Purifying EVs by spin-based size-exclusion column
- Workflow

Procedure guides

- To isolate extracellular vesicles from urine, it is recommended that users isolate extracellular vesicles from first morning urine.
- Fresh urine sample **MUST** be centrifuged at 2,000 xg for 10 minutes prior to storage or pre-clearing.
- One reaction of this kit is designed for purifying extracellular vesicles from 7.2 - 50 mL of pre-cleared urine.
- Frozen urine or a sample of urine stored at 4˚C can be also applied on ExoLutE® Exosome Isolation Kit.
- Unless otherwise noted, the procedures below must be performed at **ROOM TEMPERATURE**.
- For any larger volumes of pre-cleared urine, follow by the concentrating materials on next page.

Pre-clearing

- **For a fresh urine,**
  1. Keep the urine sample at room temperature.
  2. Mix 7.2 ml urine (or concentrated urine) with 0.8 mL **Sol U** and incubate at 37˚C for 30 minutes with continuous shaking.
  3. Centrifuge at 2,000 *xg* for 10 min then transfer the supernatant to a NEW container.

NOTES,

- **For a urine sample stored at 4˚C,** incubate in 37˚C water bath for 10 minutes, and then follow the procedure above.
- **For a frozen urine sample,** completely thaw the frozen urine in 37˚C water bath, and then follow the procedure above.

(Optional) Concentrating urine

NOTE. Use centrifugal ultrafiltration device with 100 kDa MWCO [*e.g.*, Amicon centrifugal filter device (Millipore)] and perform the procedure at room temperature.

1. Rinse the centrifugal ultrafiltration devices with distilled water before use.
2. Add appropriate volume (~ 15 mL) of pre-cleared urine to the filter devices.
3. Concentrate the fluid according to the manufacturer's instruction. It is recommended to leave 1-2 mL concentrated fluid in the insert at each concentration cycle (3,000 *xg*, 5 - 15 min). **IMPORTANT!.** Do not let membrane dried out.
4. Decant the flow through then add additional urine in the insert.
5. Repeat the steps 3 - 4 until around 2 mL of final volume of concentrated urine are left in the insert at final concentrating cycle. **IMPORTANT!.** To prevent significant loss of EVs during this step, minimize usage of filter device. .
6. Transfer the concentrated urine to a NEW 15 mL conical tube and keep it at room temperature.
7. Immediately add 2 mL of serum-free basal media such as RPMI1640 or DMEM to the insert of the concentrator. Extract EVs absorbed on the inner surface of filter by vigorous pipetting (~ 10 or more times).
8. Combine the extract and recovered concentrated urine.
9. Repeat twice steps 7 - 8.
10. **Follow the procedure in the previous section.**

Extracellular vesicle enrichment

IMPORTANT! WARM UP ALL REAGENTS AND SAMPLES to room temperature before use.

1. Add 0.25 mL of Sol A then cap the tube tightly. Invert the tube for 10 times.
2. Add 1.0 mL of Sol B then cap the tube tightly. Invert the tube for 10 times.
3. Add 4.0 mL of Sol C then cap the tube tightly. Invert the tube for 10 times.
4. Place the tube on a rocker, and then shake the tube at RT for 15 minutes.
   NOTE. Cap the tube tightly to prevent leakage while shaking.
5. Loosen the tube cap, and then centrifuge at 3,000 *xg* for 20 minutes at room temperature.
   IMPORTANT! Use a swinging-bucket rotor.
6. **Start column preparation during the step 5.**
7. Decant supernatant and leave the tube upside down on a filter paper for 1 - 2 minutes to remove residual fluid.
8. Add **300 µL of Sol R** and **COMPLETELY resuspend** the pellet by pipetting.
9. Vortex the crude EV resuspension for 30 sec and leave the suspension at room temperature for 5 - 10 minutes.

Column preparation

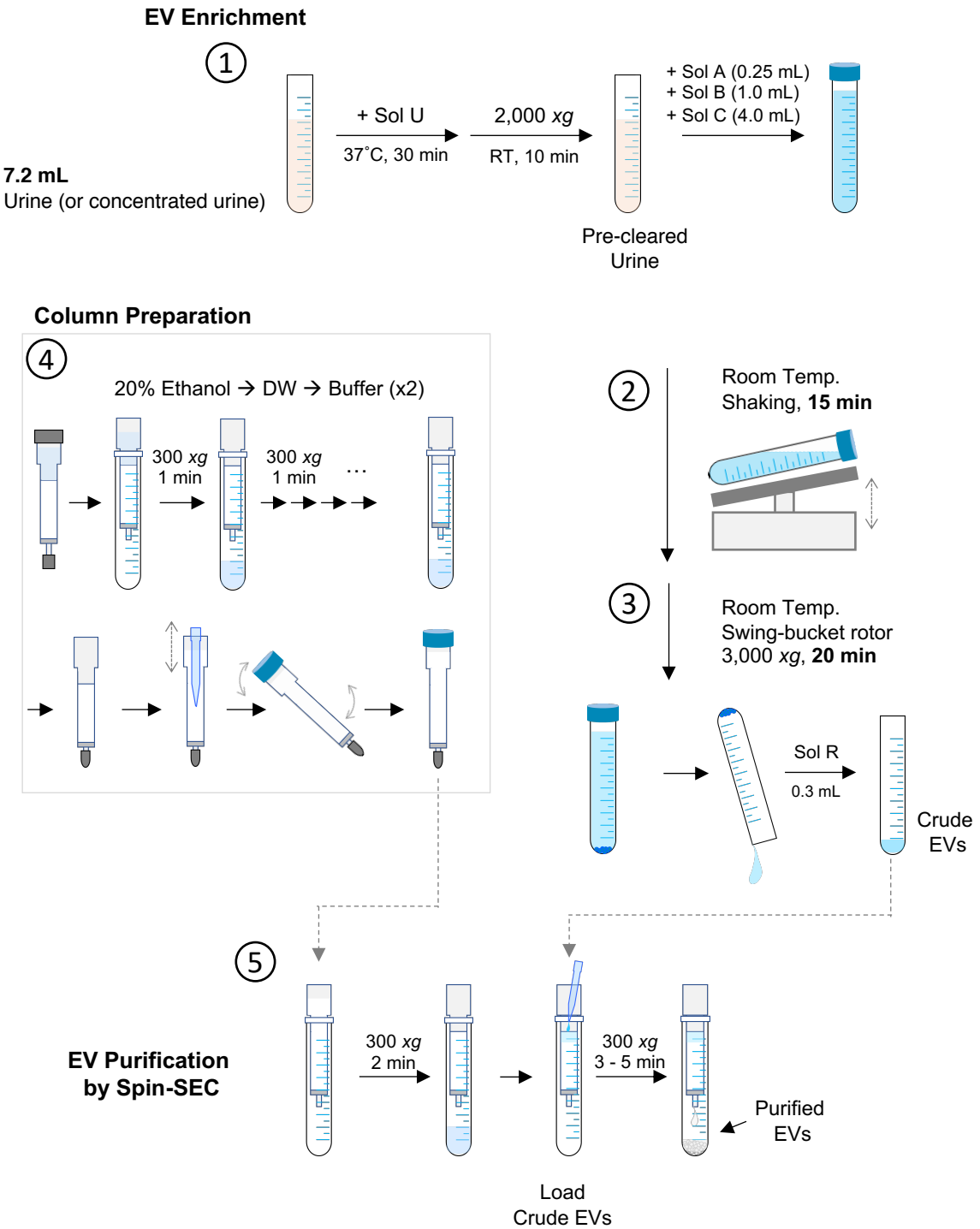
NOTE. It is **ESSENTIAL** to centrifuge the column in **a swinging-bucket** or horizontal rotor to make the sample to pass the column matrix evenly. **Set the instrument at maximum ACCELERATION and DECELERATION.**

1. Invert a Spin-SEC column several times to resuspend the white-colored matrix completely. Then stand it upright sitting in a 15 mL conical centrifuge tube (not provided).
2. Snap off the break-away end of the Spin-SEC column and remove the top cap. Place back the column in the 15 mL conical centrifuge tube.
3. Place the tube in a **swinging-bucket rotor** and centrifuge at **300 *xg* for 1 minute**.
4. Remove the flow through and replace the column in the 15 mL conical centrifuge tube.
5. Apply 2 mL of distilled water on the matrix in the column.
6. Centrifuge at **300 *xg* for 1 minute.**
7. Remove the flow through and replace the column in the 15 mL conical centrifuge tube.
8. Apply 2 mL of HEPES-buffered saline (HBS) or desired buffer on the matrix in the column.
9. Centrifuge at **300 *xg* for 1 minute.**
10. Repeat step 6 - 8.
11. Remove the flow through and replace the column in the conical tube.
12. Place a cap tightly at the end of the column. Then apply 2 mL HBS (or desired buffer or fluid) on the matrix in the column.
13. Thoroughly mix the matrix by pipetting (~ 10 times).
14. Close the top of the column then invert the column to completely degas the matrix suspension.
15. Stand the column **UPRIGHT** sitting in the 15 mL conical tube at room temperature until use.

Purifying extracellular vesicles by spin-based size-exclusion column

1. Carefully remove caps on both ends of the column prepared above then place back in the 15 mL conical tube (for waste).
2. Place the tube in the **swinging-bucket rotor** and centrifuge at **300 *xg* for 2 minutes.**
3. Transfer the column in a **NEW** 15 mL conical centrifuge tube (not provided).
4. Slowly apply **300 µL of pellet suspension** prepared in the previous section to the **CENTER** of the matrix surface.
   IMPORTANT! AVOID OVERLOADING or UNDERLOADING.
5. Stand it upright for a minute.
6. Centrifuge at **300 *xg* for 3 - 5 minutes**.
7. Remove the column. Then transfer the collected highly purified extracellular vesicles to a NEW container.
8. (Optional) It is recommended to filter the purified EVs by **0.45 µm filter devices** (centrifugal filter device is better for recovery) for downstream analysis and application.
   NOTE. For long-term storage, flash freeze aliquots of the purified extracellular vesicles in **liquid nitrogen** then store at **-60˚C or below**.

Workflow


EV Enrichment
1
7.2 mL
Urine (or concentrated urine)
+ Sol U
37˚C, 30 min
2,000 xg
RT, 10 min
Pre-cleared Urine
+ Sol A (0.25 mL)
+ Sol B (1.0 mL)
+ Sol C (4.0 mL)
Column Preparation
4
20% Ethanol → DW → Buffer (x2)
300 xg 1 min
300 xg 1 min
2
Room Temp.
Shaking, 15 min
3
Room Temp.
Swing-bucket rotor
3,000 xg, 20 min
Sol R
0.3 mL
Crude EVs
5
EV Purification by Spin-SEC
300 xg 2 min
300 xg 3 - 5 min
Purified EVs
Load Crude EVs

Products currently available from Rosetta Exosome Inc.

Table 2 ExoLutE® Exosome Isolation Kits available from Rosetta Exosome Inc.

Recommended Kit	Catalog Number	Units/Kit
ExoLutE® Conditioned Medium	EX-01	10
ExoLutE® Urine	EX-02	10
ExoLutE® Plasma & Serum	EX-03	10

Table 3 Nanoparticle-free fetal bovine serum available from Rosetta Exosome Inc.

Recommended product	Catalog Number	Unit
Nanoparticle-free fetal bovine serum	ES-01	50 mL

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