Exosome Isolation Kit from Urine

Cat#: Exo-UT50

EVOMIC SCIENCE LLC
725 San Aleso Ave, Suite 3, Sunnyvale, CA 94085

Evomic Science

User Instruction

ExoEZ™

Density-based Exosome Isolation Kit from Urine

Cat#: Exo-UT50

Store kit at +4°C to room temperature on receipt

General Tips for Exosome Isolation

- All biofluids should be considered biohazards and should be disposed according to the researcher's institution, state and federal regulation.
- Personal Protective Equipment should be worn at all the time when working on biofluids.
- Since different biofluids have highly variable compositions, the specifically optimized sample processing for each type of biofluids is required.
- Sample collecting and handling prior to purification can have a significant impact on the purity and yield of isolated exosomes! (Clotilde Théry *et al 2018 Journal of Extracellular Vesicles*.)
- In all processing steps from biofluids, consideration should be taken to prevent lysis of cells. Intracellular vesicles due to cell lysis or platelet activation in plasma case would definitely contaminate your exosome samples. It could result in misleading conclusion.
- If biofluids will not be used immediately, any cells in biofluids must be removed prior to store at -80°C.

Exosomes in Urine

Urine is the most noninvasive, easy samples to obtain in large volumes for biomarker measurement. Pisitkun et al 2004 initially isolated and described urine exosomes, which, in addition to the common exosome molecular markers, contained urinary tubule specific proteins and microRNAs. In Fig.1, the extracellular vesicles and miRNA distribution in urine was showed. However, the presence of nano- to micro-scale particles in urine, such as salt crystals, cells and virus, severely interferes with urine sub-fraction and downstream exosome isolation. One of the most abundance proteins in urine, Tamm–Horsfall protein (THP), is the main contributor to this problem. THP molecules exist not only as a mono protein molecule but also as polymerized micron long fibrils. These linear fibrils further form a three-dimensional gel which entraps a large amount of exosomes. In classical urine assay, urine is separated into various fractions by the differential centrifugation. Our data demonstrate that even low (2,000g) speed centrifugation co-precipitates cells and salt crystals with THP gel which entrapped exosomes. This low-speed pellet is usually discarded during exosome isolation procedures, as reported in the current literatures and commercial kits. However, this low-speed pellet contains around 40% of total urine exosomes. Given the fact that urine has very low exosome load, the exosomes in this low-speed pellet thus have to be released to increase total urine exosome yield. Several labs utilized the reducing agent DTT or the detergent 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonic (CHAPS) to break down the urine THP gel to release exosomes. DTT and CHAPS treatment did not only break down urine THP gel, but also damaged the integrity of cells and exosomes, and thus resulted in the inaccurate measurement and misleading discovery of proteins and miRNAs.

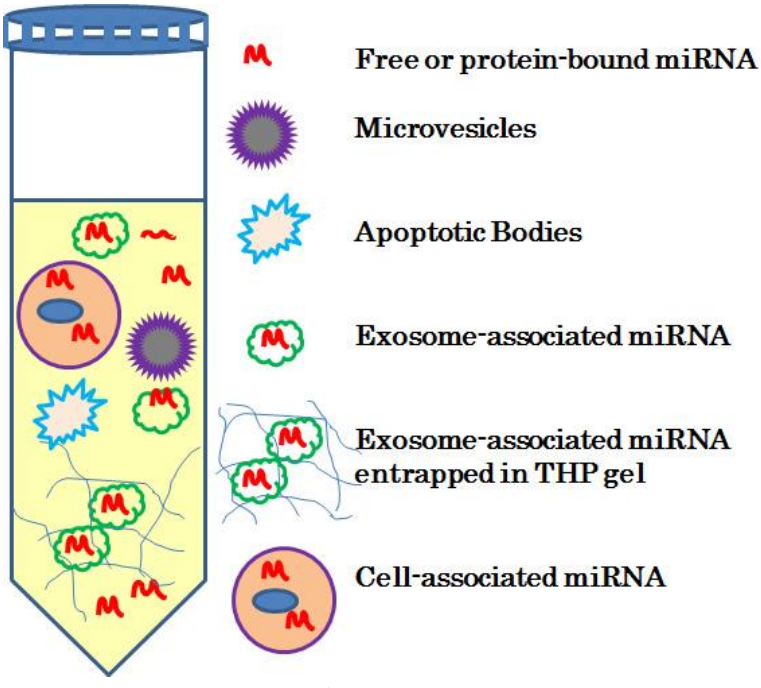

Fig.1, the extracellular vesicle and miRNA distribution in urine.

We thus developed a **ME buffer,** which efficiently released the entrapped exosomes from THP gel, as well as kept the integrity of exosomes and cells. After treated with **ME buffer**, urine exosomes can be concentrated with the **density-based** ExoEZ™ exosome isolation kit within less than 30 minutes**.** The isolated exosomes are suitable for most of applications nowadays. To remove the trace reagents and contaminated proteins, the exosomes can be further purified through the Exosome Purification Column kit (Cat# Exo-A300 or Exo-B300 or Exo-C300).

A procedure for urine exosome isolation is outlined below.

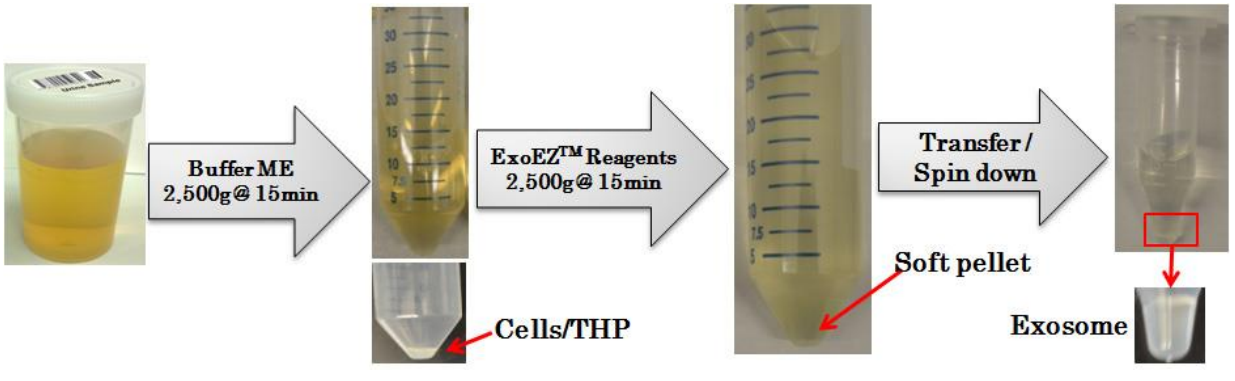

Fig. 2. A brief procedure for urine exosome isolation. Fresh raw urine (20ml) is treated with ME buffer for 15~30 min at room temperature and then centrifuged at 2,500g for 10 min. The supernatants was transferred to a new tube and mixed with ExoEZ™ exosome isolation reagents. After centrifuged at 2,500g

for 15 min, the pellet contains exosomes. Transfer this soft pellet to a new microtube and spin down at 2,500g for 3~5 min, remove trace supernatants, the pellet is exosome.

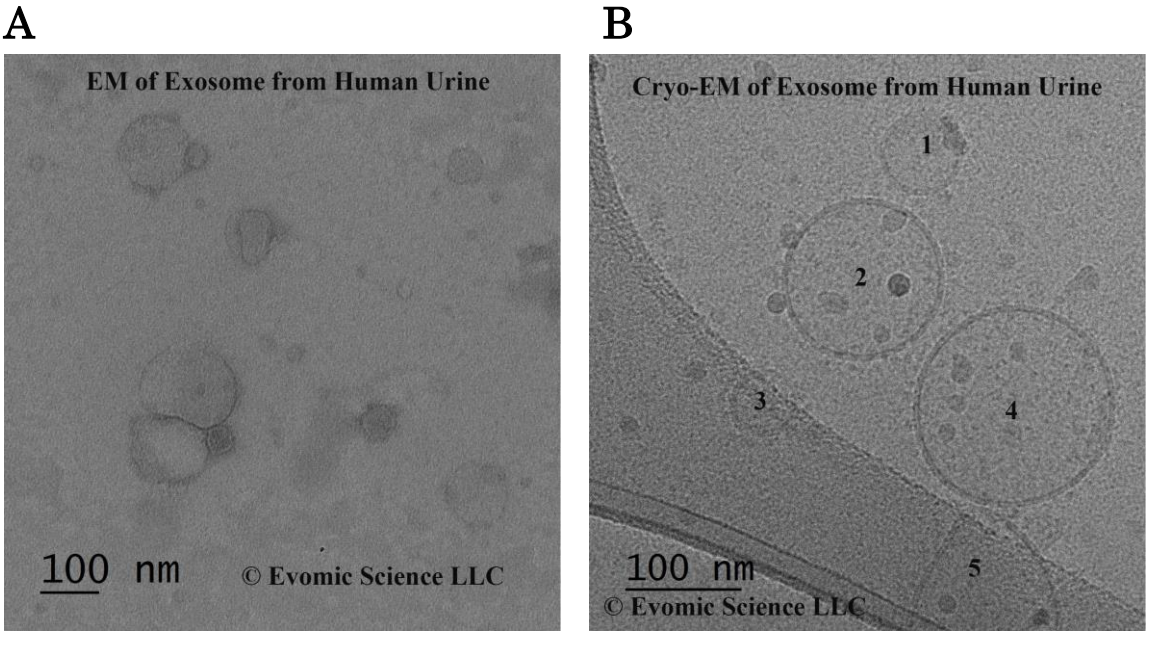

Fig. 3. TEM and Cryo-EM of purified exosomes. Exosomes were isolated from 20 ml of human urine using ExoEZ™ Exosome Isolation Kit (Cat#: ExoUT50), re-suspended in 200 µl of PBS, and then contrasted with uranyl-acetate and digital images were captured by using TECNAI (in the **A** panel). A cryo-EM images of exosome from the same urine sample were also captured (in the **B** panel), which showed the five different sizes of extracellular Vesicles.

Kit Components of Exo-UT50 (Suitable for up to 50 ml of urine)

Components	Volume
Buffer ME	5 ml
Buffer P1	25 ml
Buffer P2	25 ml
Buffer D	0.5 ml
Buffer F	0.5 ml

Urine Collection

1. Prior to urine collection, cleaning of the urogenital area with clean wipe is recommended.
2. To avoid large variation of exosome amount between samples, the **second morning urine** is recommended for urine exosome isolation.

3. Collect midstream urine in a fresh standard urine collection container.
4. Add protease inhibitors and preservatives to urine, especially if urine exosomes would be used for proteomics.
5. All urine samples should be processed fresh within 4 hours from their collection to avoid the precipitation formation, which contains exosomes. If not, urine samples should be processed to remove cells, and then stored in -80°C for longer periods.
6. The exosome concentration in urine samples is highly variable, among different subjects and also in different samples of the same subject at the certain time point and status. A normalization would be essential to compare exosome yield, especially for biomarker discovery and validation. Several normalization methods, such as time normalization, creatinine normalization, and protein normalization, can be used to normalize exosome yield but with the various advantage and disadvantage.
7. Please save some unprocessed urine sample and the different pellets and supernatants in exosome isolation procedures, if possible. It might be useful for future systemic study and normalization.
8. Avoid repeated thaw and freeze cycles.

Urine Sample Preparation

1. Transfer the desired volume of fresh urine to a new tube.
2. To release the entrapped exosome in THP gel, Add 0.1 (10%) volume of ME buffer into urine samples (such as 1 ml of ME buffer to 10 ml of urine sample) and mix well (Do not vortex).
3. Incubate at room temperature for 15~30 minutes.
4. Centrifuge the treated urine samples at 2,500g for 15 minutes at 4˚C to remove cells and salt crystals. This cell pellet can be used for other bioassays, if desired.
5. Carefully transfer supernatant into new tubes and then go through 0.22 μm filter or centrifuge supernatants at 17,000g for 10 minutes at 4˚C to remove most of large microvesicles, if possible.

Exosome Isolation

1. Transfer the desired volume of the above processed urine to a new tube and add 0.5 volumes of Buffer P1, 0.5 volumes of Buffer P2, and1/100th volume of Reagent D and F, respectively.

Processed Urine	Buffer P1	Buffer P2	Buffer D	Buffer F
5 ml	2.5 ml	2.5 ml	50 µl	50 µl
10 ml	5 ml	5 ml	100 µl	100 µl

2. Mix supernatants with the exosome isolation reagents well (Do not vortex), and then centrifuge the samples at 2,500g for 15 min at 4°C.
3. After centrifugation, discard supernatants carefully with pipette. Do not touch the soft phase in the bottom!
4. Transfer the soft phase with supernatants (200~300 µl) to a 2 ml dolphin microtube.
5. Spin down for 3~5 min at 2,500g.
6. Exosomes are concentrated on the interface and bottom phases! Remove the extra reagents / supernatants carefully with pipette! Do not touch the interface and bottom phases!
7. Suspend the concentrated exosomes in 50 µl~300 µl of PBS or your buffer.
8. These exosomes are suitable for most of applications, such as RNA isolation, ELISA and western blot, *in vitro* loading of RNAs, and *in vivo* animal study.
9. If purer exosomes (such as for Protein Mass Spectrometer) are desired, exosomes should be further purified by the Exosome Purification kit (**Cat# ExoA300, or ExoB300, or ExoC300**) or immunoaffinity beads, to remove trace contaminated proteins and precipitation reagents.
10. We recommend to use the fresh isolated exosomes immediately. Otherwise please store at 4˚C for overnight, or freeze at -20˚C or -80˚C for longer periods. Note that repeated thaw and freeze cycles can lead to some loss of exosomes.
11. When urine exosomes are used for RNA isolation, **Do not use classical TRIZOL reagent for miRNA isolation**. Using *Quick*-RNA Mini or Microprep Kit from Zymo Research or mirVana miRNA isolation kit from Thermofisher will give a good result, when elution buffer was 95°C RNase-free H_2O.