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ExoPure™ Isolation Kit (Urine)

(Catalog # K1240-2, -10; Store at RT)

I. Introduction:

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both the amount and molecular composition of the released exosomes depend on the state of a parent cell. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

ExoPure™ Isolation Kit (Urine) is a fast and convenient method of exosome isolation and purification at high yields from urine. This kit yields highly pure exosomes using filtration method as compared to exosome precipitation methods.

II. Applications:

- Easy to use: No ultra-centrifugation (<2 hr)
- 10-fold higher yield as compared to other kits or ultracentrifuge method
- Cost effective as compared to antibody bead method
- **Isolates pure exosome (exosome purity >95%)**
- Intact exosomes (good morphology)
- Use as little as 1 mL urine to achieve high yield of exosomes for any downstream applications: EM study, exosome label, exosome subpopulation, qRT-PCR profiling of exosomal miRNAs, and gel analysis of exosomal proteins
- The yield of each reaction using this kit is 50~200 µL exosome, from which 150~400 µg exosomal protein or 50~200 ng exosomal RNA can be extracted
- Easy to store and ships at room temperature (RT)

III. Sample Type:

- Urine

IV. Kit Contents: (exosome Isolation from urine):

Components	K1240-2	K1240-10	Part Number
	2 reactions	10 reactions	
Solution A (Blue)	0.5 mL	2.5 mL	K1240-XX-1
Solution B	0.5 mL	2.5 mL	K1240-XX-2
Solution C	2 mL	10 mL	K1240-XX-3
ExoPure™ Column	2	10	K1240-XX-4

V. User Supplied Reagents and Equipment:

- Glass tubes

VI. Shipment and Storage:

- ExoPure™ Isolation Kit is shipped at room temp. Keep all the bottles upright in a cool and dark place for up to 12 months. DO NOT FREEZE!

VII. Reagent Preparation and Storage Conditions:

- Cap all the bottles well immediately after each use, to prevent evaporation.
- Use glass tubes instead of plastic tubes.
- Because the exosome yield of this kit is 10 fold higher than all other kits, you can start with only 1 mL urine.
- One ExoPure™ Column is only for one reaction. **Do not exceed 3 mL urine to avoid indistinct layer separation and column clogging.**

VIII. ExoPure™ Assay Protocol:

Urine Volume	Total Volume (A+B+C)	Solution A	Solution B	Solution C
1 mL (minimum)	0.5 mL	0.083 mL	0.083 mL	0.334 mL
2 mL	1.0 mL	0.166 mL	0.166 mL	0.668 mL
3 mL (maximum)	1.5 mL	0.25 mL	0.25 mL	1.0 mL

Maximum urine volume per reaction is 3 mL. Processing more than 3 mL urine may cause indistinct layer separation and column clogging.

IX. Sample Preparation for Exosome Isolation (processing 2 mL urine sample):

1. Centrifuge 2 mL urine sample at 3,000g for 15 min at 4ºC to remove cells and debris. *Important: skip this step may cause filter clog in step 15*.
2. Without disturbing pellets, transfer 2 mL clear supernatant to a new glass tube 1 and keep it on ice.
3. In a new glass tube 2, add Solution A/B/C in the following order to prepare mixture A/B/C:
1st Solution A (blue) (0.166 mL); 2nd Solution B (0.166 mL); 3rd Solution C (0.668 mL). *Cap all Solutions A, B, C bottles well immediately after each use to prevent evaporation. When process different volume of urine, please refer to "Reaction Volume Table" for the solution A/B/C recipe.
4. Vortex the glass tube 2 (mixture A/B/C) for 10 sec to obtain a homogenous solution.
5. Transfer all mixture A/B/C (1 mL) from glass tube 2 to glass tube 1 (to mix with the 2 mL urine).
6. Cap glass tube 1 well, vigorously vortex for 30 sec, then incubate at 4°C for 1 hr
7. a. The mixture now appears as 3 layers (as shown in figure below): *Top blue layer; **Middle fluffy layer (Exosome is in this layer)**; Bottom colorless layer.* Without disturbing the middle fluffy layer, carefully pipet out the top blue layer and discard it. Then go to step 9.

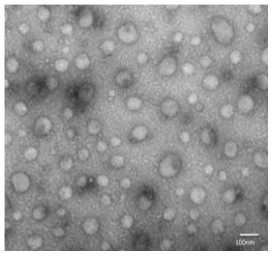
7.b. Sometimes, only two layers are visible, Top blue layer and white Cloudy layer at bottom. Carefully remove the Top blue layer and discard it. Then go to step 9. *Notice: If layer separation is indistinct, add another 1 mL mixture A/B/C (1 mL is for this example experiment), vortex for 30 sec to mix well, and incubate at 4°C for another 30 min. Then start again from step 7a.*

8. Transfer the left over in the glass tube 1 to a new 2.0 mL microcentrifuge tube (not provided in the kit) and spin at 5,000g for 3 min. A new three-layer separation will occur (Top blue layer, Middle fluffy layer and Bottom colorless layer as shown in the following figure). Proceed to next step immediately, or the layer separation may become blur.
   Top blue layer; Middle fluffy layer (Exosome is in this layer); Bottom colorless layer
9. Pipet out the Top blue layer and discard it. Insert pipette tip down to the tube bottom to remove the Bottom colorless layer completely. Only keep the Middle fluffy layer in the tube.

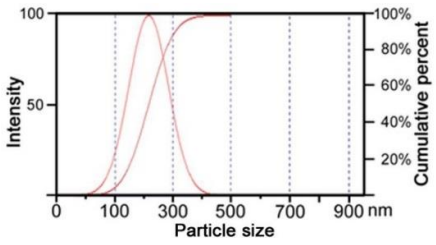
Pipet out the Top blue layer; Remove the Bottom colorless layer; ***only keep the Middle fluffy layer***

10. Transfer the whole fluffy layer to a new 0.5 mL microcentrifuge tube. Spin at 5,000g for 3 min, then repeat step 10 for one time. Now only the "fluff pellet" is left in the tube, and its volume is about 25 µL in our case.
11. Leave the tube cap open to air dry for 5-10 min at room temp. **Do not over dry.**
12. Add 1X PBS equal to 4 times volumes of the collected fluff pellet to the 0.5 mL tube. In our case, we added 100 µL PBS (4 x 25 µL fluff pellet). Resuspend the fluff pellet by pipetting up and down vigorously for 40 times.
13. Shake the 0.5 mL tube on a horizontal shaker for 3 min at high speed, then pipet up and down vigorously for 10 times. Repeat this "shake-pipet up and down" for another 2 times. *Note: This step is important. If the fluff pellet is not well re-suspended, the exosome may be trapped in the fluff pellet resulting in low exosome purity and yield. For some types of samples, it is difficult to dissociate the fluff pellet to release exosome. In such case, extend the pipetting and shaking time in step 12 and 13.*
14. Spin the 0.5 mL tube at 5,000g for 5 min. Transfer the supernatant carefully into one ExoPure™ Column (provided). Do not disturb the fluff pellet. *Note: Keep the fluff pellet at 4oC. Do not discard it until the experiment is finished. See Trouble shooting 1.2 for details.*
15. Spin the Column at 1,000g for 5 min to collect all the "flow-through".
16. **The "flow-through" is the isolated pure exosome (exosome suspended in PBS).** The whole protocol is completed here. Use the isolated exosome directly for downstream applications or store at 4°C for up to 1 week, or store at -80°C for up to 3 months. Concentrated exosome will precipitate after sitting. Pipet up and down to resuspend it well before each use.

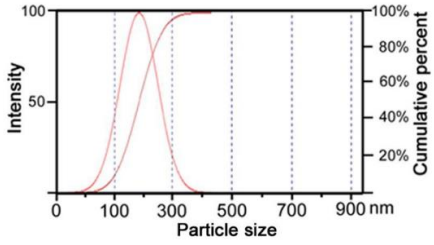
X. Sensitivity:



Transmission electron microscopy (TEM) micrography of exosomes recovered from NIH3T3 cell media with ExoPure™ Isolation kit. Isolated exosomes show spherical and membrane encapsulated particles with the diameters varying between 20-200 nm. Homogeneous Spherical Exosome Isolated by ExoPure™ Isolation Kit (EM Analysis).

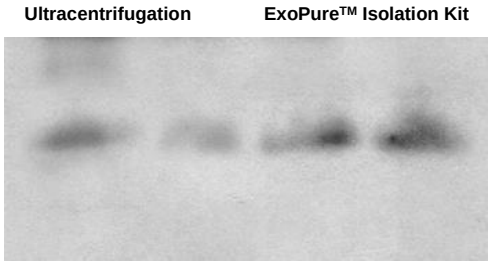


Ultracentrifugation

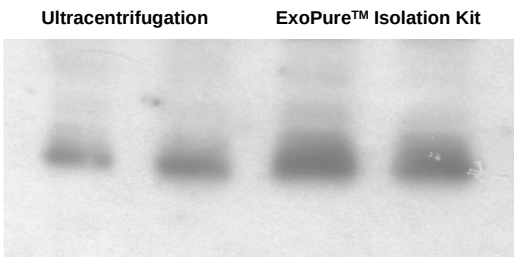


ExoPure™ Isolation Kit

Representative dynamic light scattering (DLS, 632.8 nm laser) number distribution measurement of isolated exosome population from NIH3T3 cells (1x10^6 cells) demonstrates a single peak (~150 nm) diameter. Homogenous Size Distribution of Exosomes Isolated by ExoPure™ Kit.

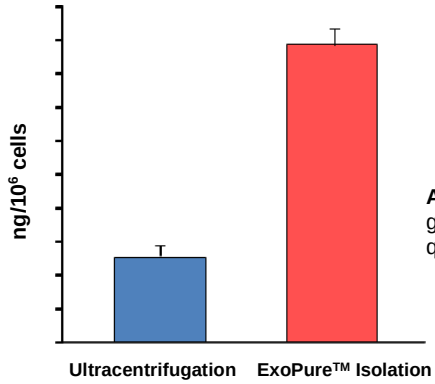


CD9



Flotillin-1

Immunoblots for exosomal marker CD9 and lipid-raft marker Flotillin-1 in cell culture medium of cultured NIH 3T3 cells. Exosomes isolated by ExoPure™ Exosome Isolation kit yield protein with greater purity and quantity than ultracentrifugation, which enhances the accuracy and sensitivity to detect biomarkers carried by exosomes.



Analysis of exosomal RNA levels from NIH 3T3 cell media. Exosome Isolation kit produced greater quantity of exosomal RNA than ultracentrifugation as verified by exosomal RNA quantification.

XI. Related Products:

Products/Catalog Number
ExoPure™ Isolation Kit (Cell Media) # K1237-2, -10
ExoPure™ Isolation Kit (Serum, Plasma) # K1238-2, -10
ExoPure™ Isolation Kit (Stem Cell Media) # K1239-2, -10
ExoPure™ Isolation Kit (Urine) # K1240-2, -10
ExoPure™ Isolation Kit (Bio Fluids) # K1241-2, -10

XII. Trouble Shooting:

1. **The final exosome yield is low.**
   - **a.** Check if there is left over liquid in the column. If yes, it indicates the column is clogged by contaminated protein. Several reasons could cause the clogging, such as cell debris was not removed completely in step 2; serum was added in the medium; some precipitation was pipet up in step 14; too much sample was loaded, etc. If this clogging happens, prepare the sample again, input lower amount of sample, and pay more attention in step 2 and 14.
   - **b.** For some type of sample, the fluff (in step 13) is very difficult to be resuspended, and the exosme may be trapped in the fluff. This can be examined by check exosome marker level in step 14 pellet and the final exosome flow-through using ELISA. If the signal from step 14 pellet is high, the exosome release step is incomplete. Add the final flow through back to the fluff pellet stored in 4°C (in step 14), pipet up and down vigorously 60 times, and shake the tube on a horizontal shaker for 20 min. Repeat pipetting up and down vigorously a few times in the middle. Go through another column to collect the exosomes.
   - **c.** For some cell type, the production of exosome is low. Generally, the cells produce more exosomes when they are in fast proliferating phase. Tune the cell culture condition (seeding density, splitting intervals etc.) to achieve optimal cell growth condition to collect more exosome. Also increase the initial input medium volume to collect more exosome.
2. **The flow through has multiple layers.** There was bottom and/or top layer left in the fluff during step 9~11. Spin the tube at 5,000g for 3 min, and carefully pipet out the top and bottom layer. Pass the sample through a new column to collect the flow through.
3. **Exosome yield is good, but exosomal protein level is low.** Exosome membrane is more difficult to be lysed than cells. Lysis buffer for cells, such as RIPA, is not able to lysis exosome to release exosomal protein.
4. **Exosome yield is good, but exosomal RNA level is low.**
   - **a.** RNA degradation. Please check the working environment for RNase free.
   - **b.** Also can add spike-in RNA to isolated exosome and then do RNA isolation to control the RNA extraction procedure.
5. **Exosomal RNA yield is good, but cannot get RT-PCR amplification.**
   - **a.** Please check internal control amplification.
   - **b.** Please check the primer sensitivity.

XIII. Important notes regarding ExoPure™ Isolation Kit:

- Our kit cannot isolate vesicles bigger than 300 nm.
- K1240 kit can be used to isolate exosome from Urine but not for isolating microparticles from cells.
- K1240 do not use antibody or beads for the isolation. So the final exosome prep is not contaminated with artificial IgG or beads.
- The final isolated exosomes are aggregated nanoparticles suspended in PBS (containing a very small amount of original culture medium or serum). The suspension buffer is compatible with most of the downstream assays, including RNA/protein extraction, TEM assay, surface labeling, etc.
- To examine that the exosome prep is pure, free of other membrane-derived microparticles (shredding vesicles: TEM assay can be used. The isolated microvesicle from cell culture medium or serum using our isolation kits showed sphere membrane encapsulated particles with the diameters varying between 20~200 nm under EM scanning. These characters help to determine that the harvested microvesicle are exosomes. Shedding particles are reported to be irregular shape with diameter up to 1000nm. In addition, the size distribution assay by dynamic light scattering can be used. The size distribution pattern of our isolated microvesicles are of diameter between 100 and 200 nm.
- Exosomes isolated using ExoPure™ Isolation Kit is functionally active: Function of specific miRNAs carried by our kits isolated exosomes were examined. Luciferase activity assay showed active function of exosomal microRNA after the exosomes were administrated to cells transfected with targeting mRNA 3'UTR vector.

FOR RESEARCH USE ONLY! Not to be used on humans.