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ExoPure™ Isolation Kit (Stem Cell Media)

(Catalog # K1239-2, -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 (Stem Cell Media) is a fast and convenient method of exosome isolation and purification at high yields from stem cell culture media. 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)
- Each reaction can process 20 mL stem cell culture medium. The yield of each reaction can yield pure exosome suspended in 50~200 µL PBS.
- Isolated exosomes are suitable for a wide range of downstream analyses, such as EM study, exosome label, exosome subpopulation, qRT-PCR profiling of exosomal miRNAs, and gel analysis of exosomal proteins western blotting
- Easy to store and ships at room temperature (RT).

III. Sample Type:

- Stem Cell Media

IV. Kit Contents: (exosome Isolation from stem cell media):

Components	K1239-2	K1239-10	Part Number
	2 reactions	10 reactions	
Solution A (Blue)	1.5 mL	7.5 mL	K1239-XX-1
Solution B	1.5 mL	7.5 mL	K1239-XX-2
Solution C	6 mL	10 mL x 3 bottles	K1239-XX-3
ExoPure™ Column	2	10	K1239-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.
- The maximum medium volume of each reaction is 20 mL from at most $1x10^7$ cells. Do not exceed the suggested sample volume or the cell number. Otherwise it may cause indistinct layer separation and column clogging.
- One ExoPure™ Column is only for one reaction.

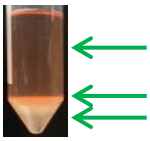
VIII. ExoPure™ Assay Protocol:

Sample Preparation for Exosome Isolation:

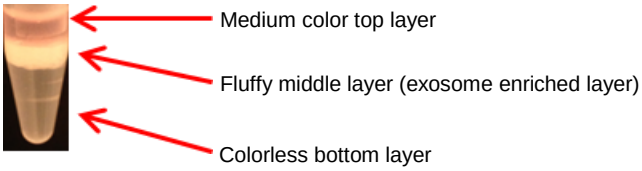
Fetal bovine serum (FBS) contains high level exosomes which may contaminate the cell derived exosomes. Use serum-free conditioned media to **starve stem cells for 48 hr before media harvest.**

1. Collect 20 mL cell culture medium.
2. Centrifuge the cell media at 3,000g for 15 min at 4°C to remove cells & debris. *Imp: skipping this step may cause filter clog in step 14.*
3. Transfer 20 mL clear supernatant (cell-free culture media) to a new 50 mL centrifuge tube (tube 1) and keep it on ice. **The maximum medium volume of each reaction is 20 mL from at most $1x10^7$ cells. Do not exceed the suggested sample volume or the cell number. Otherwise it may cause indistinct layer separation and column clogging.* ***One Column can be used for only one reaction.***
4. In another 50 mL centrifuge tube (tube 2), add the solutions in the following order to prepare A/B/C mixture (always prepare A/B/C mixture right before use): 1st Solution A (0.75 mL); 2nd Solution B (0.75 mL); 3rd Solution C (3 mL). *Cap Solution A, B & C bottles immediately after each use.
5. Vortex the tube 2 (4.5 mL A/B/C mixture) for 5~10 sec to obtain a homogenous solution.
6. Add the 4.5 mL A/B/C mixture from tube 2 to tube 1 (20 mL cell-free culture media).
7. Tightly cap tube 1, vigorously vortex for 30 sec, and then incubate at 4°C for 30 min.

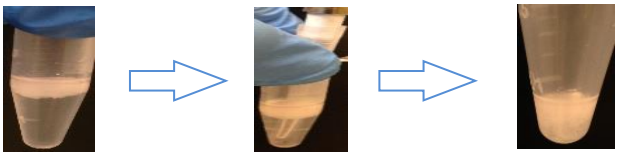
8a. The mixture now appears as 3 layers: Top layer, medium color; *Bottom layer, colorless;* ***Middle fluffy layer (exosome enriched layer).***



Without disturbing the middle fluff layer, carefully aspirate the top layer using a pipette and discard it, and then go to step 9.
8b. Occasionally, only two layers (medium color top layer and white cloudy/fluffy layer) are visible. Remove and discard the top layer, and then go to step 9. *Notice: If no layer separation was clearly seen at all, add another 1 volume (4.5 mL in this example) of solution A/B/C mixture, mix well, and incubate for another 30 min in 4°C. Proceed as step 8a described.*
9. Transfer the left over in the tube to a new 50 mL centrifuge tube (not provided) and spin at 5,000g for 3 min. A new three-layer separation will occur. Proceed to next step immediately. *Medium color top layer;* ***Fluffy middle layer (exosome enriched layer)****; Colorless bottom layer.*



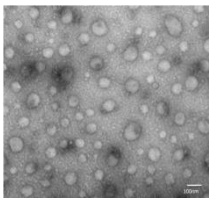
10. Pipet out and discard the top layer. Insert the pipette tip down to the tube bottom to remove the colorless bottom layer completely. **Only keep the fluff middle layer in the tube.**



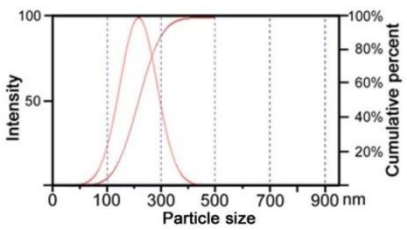
Pipet out the Top transparent layer | Remove the Bottom layer | **Only keep the Middle fluffy layer**

11. Transfer the "fluff" to a new 1.5 mL microcentrifuge tube, and repeat spinning at 5,000g for 3 min and repeat step 10 once.
12. Leave the tube cap open to air dry for 5~10 min at room temp. **Do not over dry.**
13. Add 1X PBS as much as 1-2 volumes of the collected fluff to the tube. Resuspend the "fluff" by pipetting up and down vigorously for 40 times. Shake the tube on a horizontal shaker for 10 min at high speed. In the middle, repeat pipetting up and down vigorously a couple of times. ***Note: This step is important. If the fluff is not well re-suspended, the exosome may be trapped in the fluff and the final yield will be low. For some type of samples, the pellet is sticky and difficult to be dissociated, from which the exosome is difficult to be released. Extend the pipetting and shaking time as needed. To examine if the exosome is trapped in the fluff precipitation, check exosomal 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.***
14. Spin the tube at 5,000g for 5 min. Transfer the supernatant carefully into ExoPure™ Column (provided). Do not disturb the pellet. *Note: Keep the fluff pellet at 4°C. Do not throw it until the experiment is finished.*
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 (suspended in PBS).** 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.

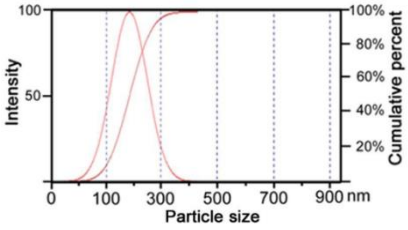
IX. Sensitivity:



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).

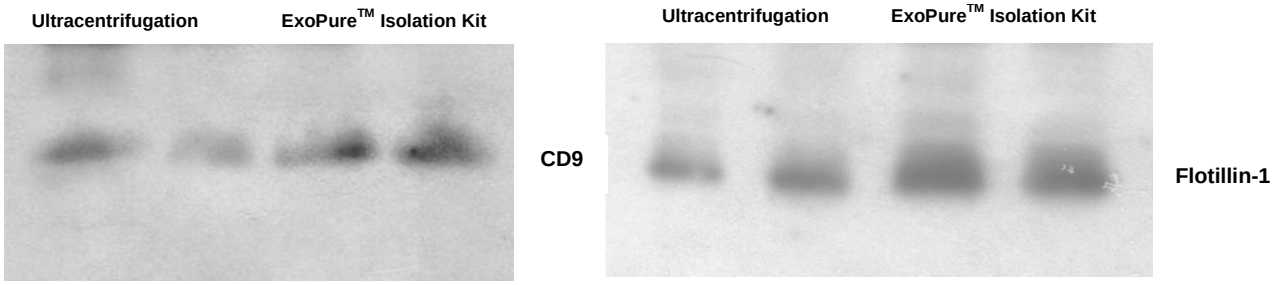


Ultracentrifugation

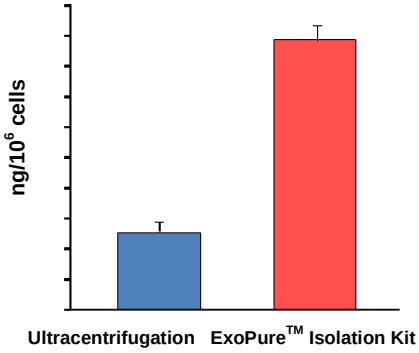


ExoPure™ Isolation Kit

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.



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.

X. 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

XI. 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 exosome 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 though.
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.

XII. Important notes regarding ExoPure™ Isolation Kit:

- Our kit cannot isolate vesicles bigger than 300 nm.
- K1239 kit can be used to isolate exosome from stem cell media but not for isolating microparticles from cells.

- K1239 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 1000 nm. 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.