ExoGenie Exosome Isolation Kit (Cell Media) (BN00097)

(Catalog # BN00097 -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.

Isolation Kit (Cell Media) is a fast and convenient method of exosome isolation and purification at high yields from cell culture media. This kit yields much highly pure exosomes as compared to exosome precipitation methods.

II. Application:

- Easy to use: No ultra-centrifugation (<2 hr)
- 10-fold higher yield as compared to other kits or ultracentrifuge methods
- Cost effective as compared to antibody bead method
- Isolates pure exosome ***(exosome purity >95%)***
- Intact exosomes (good morphology)
- Small sample volume as little as 2 mL of cell media to isolate high yield of exosomes
- 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:

- Cell culture media

IV. Kit Contents: (exosome Isolation from 2 mL cell media):

Components	BN00097	BN00097	Part Number
	2 reactions	10 reactions	
Solution A (Blue)	0.5 mL	2.5 mL	BN00097-1
Solution B	0.5 mL	2.5 mL	BN00097-2
Solution C	2.0 mL	10.0 mL	BN00097-3
ExoGenie Column	2	10	BN00097-4

V. User Supplied Reagents and Equipment:

- Glass tubes

VI. Shipment and Storage:

- ExoGenie 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.
- Each reaction can process 2~4 mL cell culture medium. Each reaction can yield pure exosome suspended in 50~200 µL PBS (from which 150~400 µg exosomal protein or 50~200 ng exosomal RNA can be extracted. The maximum medium volume of each reaction is 4 mL from at most $5x10^5$ cells.
- One ExoGenie Column is only for one reaction. Do not exceed 4 mL of cell medium or $5x10^5$ cells to avoid indistinct layer separation and column clogging.

VIII. ExoGenie (Exosome Isolation from cell media) Assay Protocol:

- Fetal bovine serum (FBS), even the so-called **"exosome-free FBS"** contains high level exosomes that will contaminate the cell derived exosomes. Use serum-free media to starve the cells for 48 hr before the media harvest.
- If the cultured cells are highly proliferative, such as tumor cells, dilute the cell culture medium by 1:2 first.
- If the culture medium is from bioreactor system, dilute the medium to no more than $2x10^5$ cells/2 mL.

1. Collect 2 mL cell culture medium.
2. Centrifuge the cell culture media at 3,000g for 15 min at 4°C to remove cells and debris. ***Important: skip this step may cause clogging of filter.***
3. Transfer 2 mL clear supernatant (cell-free culture media) to a new glass tube 1 and keep it on ice.
4. In glass tube 2, add Solution A/B/C in the following order to prepare mixture A/B/C (always prepare mixture A/B/C right before use): **1st add Solution A (0.125 mL); 2nd add Solution B (0.125 mL); 3rd add Solution C (0.5 mL)**
   ** Tightly cap all bottles immediately after each use to prevent evaporation. When processing different volume of cell medium, please refer to "Reaction Volume Table" below for solution A/B/C recipe.*

Cell Culture Media (clear supernatant)	Total Volume (A+B+C)	Solution A	Solution B	Solution C
2 mL (minimum)	0.75 mL	0.125 mL	0.125 mL	0.5 mL
3 mL (minimum)	1.125 mL	0.187 mL	0.187 mL	0.75 mL
4 mL (minimum)	1.5 mL	0.25 mL	0.25 mL	1.0 mL

The maximum medium volume of each reaction is 4 mL from at most 5x10^5 cells. One ExoGenie Column can be used for only one reaction. Do not exceed the suggested sample volume or the cell number. Otherwise it may cause indistinct layer separation and column clogging.

5. Vortex the tube 2 (0.75 mL mixture A/B/C) for 10 sec to obtain a homogenous solution.
6. Add the 0.75 mL mixture A/B/C from tube 2 to tube 1 (2 mL cell-free culture media).
7. Tightly cap tube 1, vigorously vortex for 30 sec, then incubate at 4°C for 30 min.

8A. The mixture now appears as 3 layers (as written below):
Top medium color layer Bottom colorless layer; ***Middle fluffy layer (exosome is in this layer);*** *Bottom colorless layer.* ***Without disturbing the Middle fluffy layer, carefully pipet out the Top medium color layer and discard it. Then go to step 9.**

8B. When only two layers appear: Top medium color layer and white cloudy layer. Carefully remove the top layer and discard it. Then go to step 9. ***Notice: If the layer separation is indistinct, add another 0.75 mL mixture A/B/C (0.75 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 8A.***

9. Transfer the left over in the glass tube 1 to a new 1.5 mL microcentrifuge tube (not provided) and spin at 5,000g for 3 min. A new three-layer separation will occur. Proceed to next step immediately. *Top medium color layer;* ***Middle fluffy layer (exosome is in this layer);*** *Bottom colorless layer*

10. Pipet out the Top medium color layer and discard it. Insert pipette tip down to the tube bottom to remove the Bottom colorless layer completely. *Pipet out the Top medium color layer------- Remove the Bottom colorless layer -------****only keep the Middle fluffy layer***

11. 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.

12. Leave the tube cap open to air dry for 5-10 min at room temperature. **Do not over dry.**

13. 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.

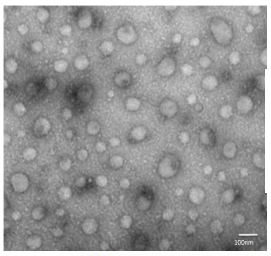
14. Shake the 0.5 mL tube on a horizontal shaker for 3 min at high speed, then pipet up and down vigorously for 10 times. Repeat this "shake-pipet up and down" for another 2 times. *Note: This step is important. If the fluff pellet is not well re-suspended, the exosome may be trapped in the fluff pellet 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 13 and 14.*

15. Spin the 0.5 mL tube at 5,000g for 5 min. Transfer the supernatant carefully into one ExoGenie Column (provided). Do not disturb the fluff pellet. *Note: Keep the fluff pellet at 4°C. Do not discard it until the experiment is finished. See Trouble shooting for details.*

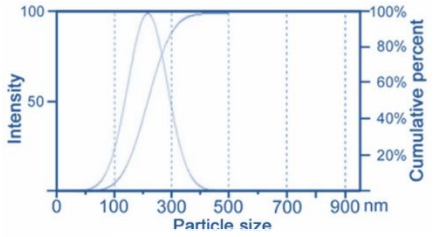
16. Spin the Column at 1,000g for 5 min to collect all the "flow through".

17. 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 (e.g.), 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.

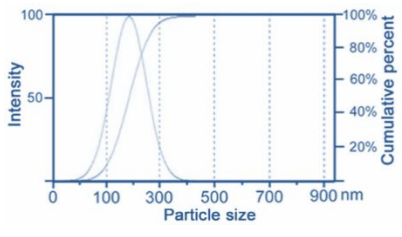
IX. Sensitivity:



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

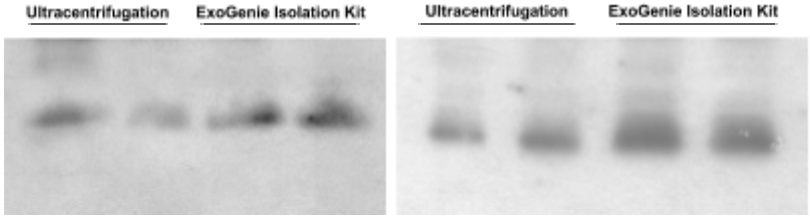


Ultracentrifugation

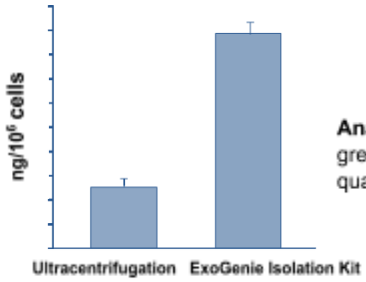


ExoGenie Isolation Kit

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 ExoGenie Kit comparable to exosomes isolated following ultracentrifugation protocols.



Immunoblots for exosomal marker CD9 and lipid-raft marker Flotillin-1 in cell culture medium of cultured NIH 3T3 cells. Exosomes isolated by ExoGenie 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. 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.

XI. Important notes regarding ExoGenie Isolation Kit:

- Our kit cannot isolate vesicles bigger than 300 nm.
- BN00097 kit can be used to isolate exosome from cell culture medium but not for isolating microparticles from cells.
- BN00097 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 ExoGenie 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.