Exosome Isolation Kit (Body Fluids)

(Catalog # BN00109 -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.

Exosome Isolation Kit (Body Fluids) is a fast and convenient method of exosome isolation and purification at high yields from body fluids including CSF, Amniotic fluid, Inflammatory fluid, Lymph fluid, Breast milk, Saliva, GI fluid, Broncho alveolar lavage fluid. This kit yields much highly pure exosomes as compared to exosome precipitation methods.

II. Application:

- Easy to use: No ultra-centrifugation (<40 min)
- 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)
- 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:

- Bio Fluids (CSF, Amniotic fluid, Inflammatory fluid, Lymph fluid, Breast milk, Saliva, GI fluid, Broncho alveolar lavage fluid)

IV. Kit Contents: (exosome Isolation from bio fluids):

Components	BN00109	BN00109	Part Number
	2 reactions	10 reactions	
Solution A (Blue)	0.5 mL	2.5 mL	BN00109-1
Solution B	0.5 mL	2.5 mL	BN00109-2
Solution C	0.5 mL	2.5 mL	BN00109-3
Sample Buffer	4 mL	20 mL	BN00109-4
Exosome Column	2	10	BN00109-5

V. User Supplied Reagents and Equipment:

- Glass tubes

VI. Shipment and Storage:

- Exosome 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.
- Use 0.5~2 mL human body fluid to achieve high yield of exosomes for any downstream applications: EM study, exosome labeling, exosome subpopulation, qRT-PCR profiling of exosomal miRNA, ELISA and gel analysis of exosomal proteins. The yield of exosome varies depending on the sample type.
- Do not process more than 2mL body fluid for each reaction. Otherwise it will cause indistinct layer separation and column clogging.
- One Exosome 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. Exosome Isolation from bio fluids Assay Protocol:

Sample Preparation:

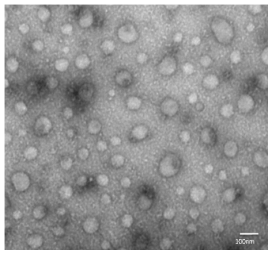
1. Collect 0.5-21 mL body fluid sample and keep it on ice. If start with frozen sample, thaw the sample completely at room temperature, and keep on ice.
2. Centrifuge the body fluid sample at 3,000g for 15 min at 4°C to remove cells and debris. *Important: skipping this step may cause filter clogging in step 15.*
3. Transfer 0.5-1 mL clear supernatant to a 15 mL centrifuge tube without disturbing the pellet. Add Sample Buffer to the supernatant to make a total volume of 2 mL diluted body fluid sample, and keep it on ice. (This dilution works well for starting sample volume between 0.5-1 mL). *Important: If starting volume of the sample is more than 1 mL, add the same volume of Sample Buffer and keep it in ice. For example, if starting with 1.2 mL saliva, add 1.2 mL Sample Buffer to dilute it. (This dilution works well for starting sample volume between 1-2 mL).*
4. In another 1.5 mL microcentrifuge tube, add Solution A/B/C in the following order to prepare 0.75 mL mixture A/B/C (always prepare mixture A/B/C right before use): 1st add Solution A (0.25 mL); 2nd add Solution B (0.25 mL); 3rd add Solution C (0.25 mL). *Cap all bottles well immediately after each use to prevent evaporation.
5. Vortex the mixture A/B/C for 10 sec to obtain a homogenous solution.
6. Add the 0.75 mL mixture A/B/C to the diluted body fluid sample (from step 3).

7. Cap the 15 mL tube, vigorously vortex for 30 sec, then incubate at 4°C for 30 min.
8. The mixture now appears as 3 layers: *Top transparent layer;* ***Middle fluffy layer (Exosome is in this layer)****; Bottom colorless layer*

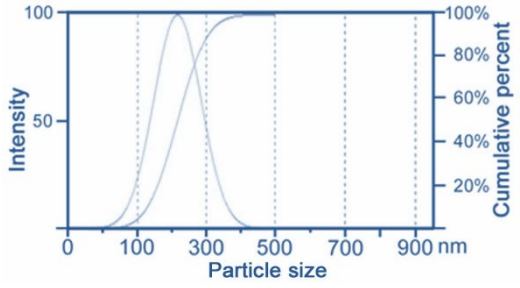
If the layer separation is not clear, centrifuge at 5,000 rpm for 3 min. Pipet out the Top transparent layer and discard it without disturbing the Middle fluffy layer.

9. Transfer the Middle fluffy layer (Exosome is in this layer) to another 1.5 mL microcentrifuge tube. Spin the tube at 5,000g for 3 min. A new three-layer separation will appear: *Top transparent layer;* ***Middle fluffy layer (Exosome is in this layer)****; Bottom colorless layer.* Proceed to the next step immediately.
10. Pipet out the Top transparent layer and discard it. Insert pipette tip down to the tube bottom to completely remove the Bottom colorless layer. **Only keep the Middle fluffy layer in the tube. Exosome is in this layer.**
11. Spin again at 5,000g for 3 min, and 3 layers will appear again. Now, repeat step 10 for one more time. Now only the "fluff pellet" left in the tube. The "fluff pellet" volume is about 25 µL in this example experiment.
12. Leave the tube cap open to air dry for 5-10 min at room temp. **Do not over dry.**
13. Add 1X PBS equal to 4 times volumes of the collected fluff pellet to the tube. In this example experiment, 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 tube on a horizontal shaker at high speed for 3 min, 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 the exosome. In such case, extend the pipetting and shaking time in step 13 and 14.*
15. Spin the tube at 5,000g for 5 min. Without disturbing the "fluff pellet", transfer the supernatant carefully into one Exosome Column (provided). *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 Exosome Column at 1,000g for 5 min to collect the "flow-through".
17. **The "flow-through" is the isolated pure exosome (exosome suspended in PBS).** The whole protocol is completed here. Use it directly for downstream assays or store at 4°C for up to 1 week, or 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.

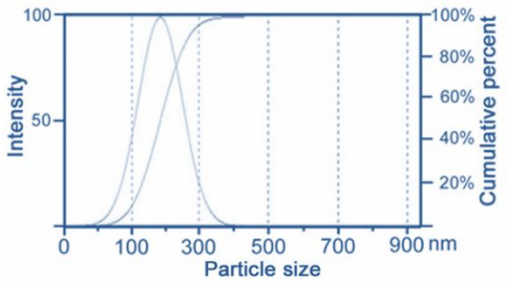
IX. Sensitivity:



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

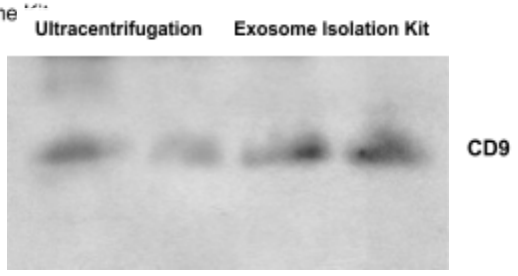


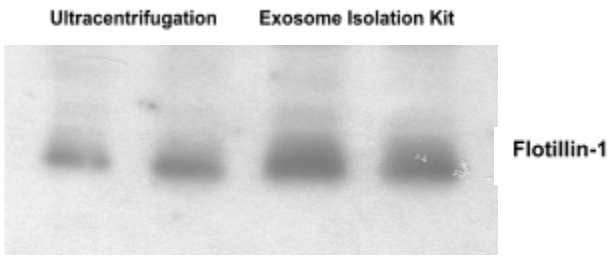
Ultracentrifugation



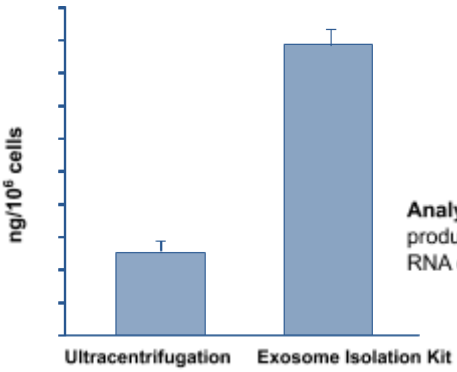
Exosome Isolation Kit

Representative dynamic light scattering (DLS, 632.8nm 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 Exosome





Immunoblots for exosomal marker CD9 and lipid-raft marker Flotillin-1 in cell culture medium of cultured NIH 3T3 cells. 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 flowthough.
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 Exosome Isolation Kit:

- Our kit cannot isolate vesicles bigger than 300 nm.
- BN00109 kit can be used to isolate exosome from cell culture medium but not for isolating microparticles from cells.
- BN00109 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 Exosome 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.