ExoGenie Exosome Isolation Kit (Serum, Plasma) (BN00099)

(Catalog # BN00099 -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.

ExoGenie Isolation Kit (Serum, Plasma) is a fast and convenient method of exosome isolation and purification at high yields from serum or plasma. This kit yields highly pure exosomes using **filtration methods** 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)
- Small sample volume as little as 100~500 µL serum or plasma. The yield of each reaction is 100~200 µL exosome, from which 300~400 µg exosomal protein or 200~300 ng exosomal RNA can be extracted. 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:

- Serum, Plasma

IV. Kit Contents: (exosome Isolation from serum, plasma):

Components	BN00099	BN00099	Part Number
	2 reactions	10 reactions	
Solution A	0.6 mL	3.0 mL	BN00099-1
Solution B	0.6 mL	3.0 mL	BN00099-2
Solution C	0.6 mL	3.0 mL	BN00099-3
Sample Buffer	4 mL	2 X 10 mL	BN00099-4
ExoGenie Column	2	10	BN00099-5

V. User Supplied Reagents and Equipment:

- Glass tubes

VI. Shipment and Storage:

- Exosome Isolation Kit is shipped and store 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 as little as 100 µL serum 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.
- **Do not process more than 500 µL serum or plasma for each reaction.** Otherwise it will cause indistinct layer separation and column clogging.
- 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 Kit Assay Protocol:

Serum Preparation for Exosome Isolation:

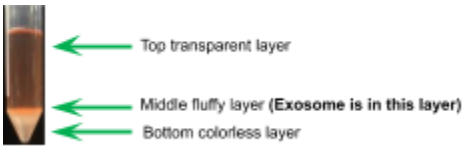
Collect whole blood in a tube. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 min. Remove the clot by centrifuging at 1,000-2,000g for 10 min in a refrigerated centrifuge. The resulting supernatant is designated as **serum**. The samples should be maintained at 2-8°C while handling. If the serum is not analyzed immediately, it should be distributed into 0.5 mL aliquots, stored, and transported at –80°C or lower. It is important to avoid freeze-thaw cycles because this is detrimental to many serum components.

Plasma Preparation for Exosome Isolation:

Collect whole blood into commercially available EDTA-treated tubes. **Never use heparinized tube because it reduces exosomal RNA activity.** Cells are removed from plasma by centrifugation for 10 min at 1,000-2,000g using a refrigerated centrifuge. Centrifugation for 15 min at 2,000g depletes platelets in the plasma sample. The resulting supernatant is designated as **plasma**. The samples should be maintained at 2-8°C while handling. If the plasma is not analyzed immediately, it should be distributed into 0.5 mL aliquots, stored, and transported at -80°C or lower. It is important to avoid freeze-thaw cycles.

Exosome Isolation Assay Protocol (Serum/Plasma):

1. Collect 100-500 µL serum or plasma sample and keep it on ice. If start with frozen sample, thaw the sample completely at room temperature, and keep it on ice.
2. Centrifuge the serum/plasma sample at 3,000g for 15 min at 4°C to remove debris. *Important: Skipping this step may cause filter clogging in step 15.*
3. Transfer 100-500 µL clear supernatant to a 15 mL centrifuge tube without disturbing the pellet. Do not process more than 500 µL sample per reaction. Add Sample Buffer to the supernatant to make a total volume of 2 mL diluted serum/plasma sample, and keep it on ice. (This dilution works well for starting sample between 100-500 µL).
4. In another 1.5 mL microcentrifuge tube, add Solution A/B/C in the following order to prepare 0.9 mL mixture A/B/C (always prepare mixture A/B/C right before use. The mixture A/B/C cannot be stored): 1st add Solution A (0.3mL); 2nd add Solution B (0.3mL); 3rd add Solution C (0.3mL). **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 0.9 mL mixture A/B/C to the 2 mL diluted serum / plasma sample (from step 3).
7. Cap the 15 mL tube, gently invert the tube for atleast 10 times to mix well, then incubate at 4°C for 30 min.
8. Spin the tube at 5000g for 3 min.
9. **A.** The mixture now appears as 3 sharp layers:

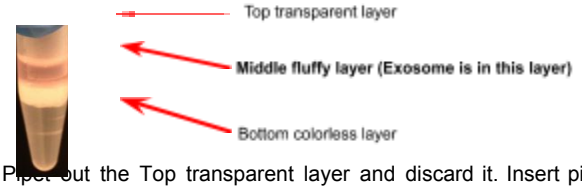


Do not disturb the Middle fluffy layer, and go to next step 10 (refer to step 9B only if 3-layer separation are not sharp).

B. For some samples, layer separation is not sharp. You can vaguely see 3 layers (as shown in the figure below): Top cloudy layer (aqueous layer), middle fluffy layer (thicker and less transparent than top layer) and bottom colorless layer.

Carefully remove the top layer and discard it. Because the separation is not sharp, be careful, not to disturb or remove the middle fluffy layer because exosome is in this layer. Prepare another 0.9 mL mixture A/B/C as described in step 4 and 5, and add it to the tube containing the middle fluffy layer and bottom colorless layer. Gently invert the tube for at least 10 times to mix well. Incubate at 4°C for another 10 min. Spin the tube at 5,000× g for 3 minutes. Now the mixture appears as 3 layers as shown in step 9a. Then go to step 10.

10. Pipet out the Top transparent layer and discard it without disturbing the Middle fluffy layer. 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.

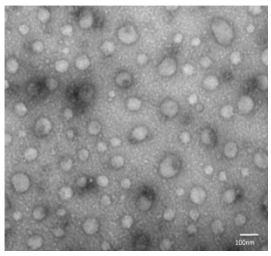


11. Pipet out the Top transparent layer and discard it. Insert pipette tip down to the tube bottom to completely remove the Bottom colorless layer. **Only keep the Middle fluffy layer in the tube. Exosome is in this layer.**

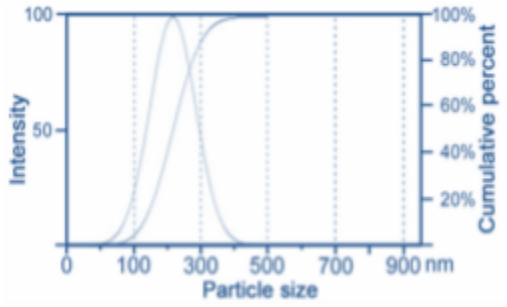
12. Spin again at 5,000g for 3 min, and 3 layers will appear again. Now, repeat step 10 for one more time. Now only the "fluff pellet" left in the tube. The "fluff pellet" volume is about 25 µL in this example experiment.
13. Leave the tube cap open to air dry for 5-10 min at room temp. **Do not over dry**.
14. 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.
15. 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, eg. Hyperlipidemia patient serum sample, 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.*
16. Spin the tube at 5,000g for 5 min. Without disturbing the "fluff pellet", transfer the supernatant carefully into one ExoGenie Column (provided). *Note: Keep the "fluff pellet" at 4°C. Do not discard it until the experiment is finished. See "Trouble shooting" for details.*

17. Spin the ExoGenie Column at 1,000g for 5 min to collect the “flow-through”.
18. **The “flow-through” is the isolated pure exosome (exosome suspended in PBS).** The whole protocol is completed here. Use it directly for downstream assays to extract exosomal RNA/Protein, 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.

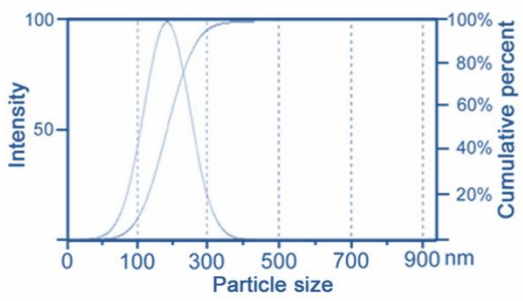
IX. Sensitivity:



Transmission electron microscopy (TEM) micrography of exosomes recovered from NIH3T3 cell media with 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).

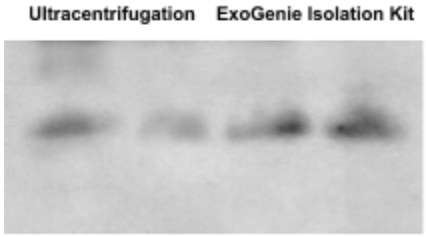


Ultracentrifugation

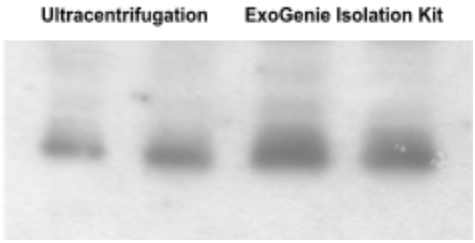


ExoGenie Isolation Kit

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.

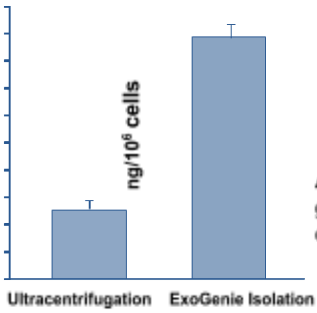


CD9



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 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 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 sample 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 ExoGenie Isolation Kit:

- Our kit cannot isolate vesicles bigger than 300 nm.
- BN00099 kit can be used to isolate exosome from serum and plasma but not for isolating microparticles from cells.
- BN00099 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 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.