Exosome DNA Extraction Kit (BN00111)

(Cat# BN00111 -25, -50; 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 DNA Extraction Kit is for exosomal DNA extraction from purified exosomes isolated by Exosome Isolation Kits . Normally, 100~300 ng of exosomal DNA can be purified from the exosome isolated from 200 µL blood serum or 5 mL cell media. The extracted DNA can be directly used for PCR and sequencing. No need to precipitate, concentrate or desalt.

II. Application:

- Easy to use
- The extracted DNA can be directly used for PCR and sequencing
- No need to precipitate, concentrate or desalt the extracted DNA

III. Sample Type:

- Cell Media, Serum, Plasma, Urine, Bio Fluids

IV. Kit Contents:

Components	BN00111	BN00111	Part Number
	25 Reactions	50 Reactions	
Buffer E1	7.5 mL	15 mL	BN00111-1
Buffer E2	7.5 mL	15 mL	BN00111-2
Buffer E3	10 mL	20 mL	BN00111-3
Buffer PN	5 mL	10 mL	BN00111-4
Buffer PS	15 mL	30 mL	BN00111-5
Buffer EB	2.5 mL	5 mL	BN00111-6
RNase A (10 mg/mL)	42 µL	84 µL	BN00111-7
Spin Column with Tube	25	50	BN00111-8

V. User Supplied Reagents and Equipment:

- Glass tubes (for exosome isolation step)
- 1.5 ml centrifuge tubes

VI. Shipment and Storage:

- Exosome DNA Extraction Kit is shipped at room temp. After adding RNase A, store Buffer E1 at 2~8°C, and it is stable for 6 months. Other buffers and RNase A stock solution can be stored for 12 months at room temperature. 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 for exosome isolation.
- All protocol steps should be carried out at room temperature (15~25°C).
- Before use, add the provided RNase A to Buffer E1. Store the “RNase added Buffer E1” at 4°C.
- All centrifugation steps are performed at 13,000 rpm in a table-top microcentrifuge at room temperature.

VIII. Exosome DNA Extraction Protocol:

1. Isolate exosomes from 200 µL serum or 5 mL cell media using our Exosome Isolation kit **(Not provided).** Resuspend the exosomes in a final volume of 100 µL PBS.
2. Add **250 µL Buffer E1** (check if RNase A has been added) to the 100 µL exosomes and mix thoroughly by vortexing or pipetting up and down till no clumps remain. *Note: If exosomes are not thoroughly mixed, the DNA yield and purity will be low.*
3. Add **250 µL Buffer E2** and gently invert the tube 4~6 times. Stand for 4~5 min at room temperature. The solution should become clear and viscous. *Note: Do not allow the lysis reaction to proceed for more than 5 min.*
4. Add **350 µL Buffer E3** and mix immediately and thoroughly by inverting the tube 4~6 times. ***Note: The white precipitate should form.***
5. Sit at room temperature for 5 min. Centrifuge for 10 min. Meanwhile place Spin Columns with tube.
6. Transfer the supernatant obtained in step 5 to Spin column with tube.
7. Centrifuge for 30~60 sec. Discard the flow through.
8. Wash the column once with **200 µL Buffer PN**. Centrifuge for 30~60 sec. Discard the flow through.
9. Wash the column once with **400 µL Buffer PS**. Centrifuge for 30~60 sec. Discard the flow through.

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10. Put the spin column back into the collection tube and centrifuge for an additional 2 min to remove residual wash buffer. *Note: The residual ethanol will affect the subsequent enzymatic reaction (digestion, PCR, etc.). Residual PS buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation.*
11. Place the spin column in a clean 1.5 mL microcentrifuge tube (not provided). Add **10~300 µL Buffer EB** (or ddH_2O) to the center of the film of each column. Let stand for 2 min at room temperature and then centrifuge for 1 min to collect flow through. The flow through is purified exosomal DNA. Use it directly or stored at -20°C.

IX. Trouble Shooting (Exosome Isolation Step):

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.

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