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ExoPure™ Isolation Kit (Body Fluids)

(Catalog # K1241-2, -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 (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.

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.
- 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	K1241-2	K1241-10	Part Number
	2 reactions	10 reactions	
Solution A (Blue)	0.6 mL	3 mL	K1241-XX-1
Solution B	0.6 mL	3 mL	K1241-XX-2
Solution C	0.6 mL	3 mL	K1241-XX-3
Sample Buffer	4 mL	20 mL	K1241-XX-4
ExoPure™ Column	2	10	K1241-XX-5

V. User Supplied Reagents and Equipment:

- 15 mL centrifuge tubes
- 1.5 mL microcentrifuge tubes
- Centrifuge
- Microcentrifuge

VI. Shipment and Storage:

- ExoPure™ Isolation Kit is shipped and stored 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:

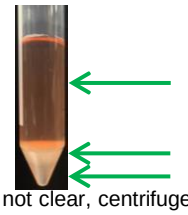
- Cap all the bottles well immediately after each use, to prevent evaporation.
- Use 0.5~2mL human body fluid to achieve high yield of exosomes for any downstream applications
- Do not process more than 2mL body fluid for each reaction. Otherwise it will cause indistinct layer separation and column clogging.

VIII. ExoPure™ (Exosome Isolation from bio fluids) Assay Protocol:

Sample Preparation:

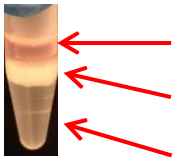
1. Collect 0.5-1 mL body fluid sample and keep it on ice. If starting with frozen sample, thaw the sample completely at room temperature, and keep on ice.
2. Centrifuge the body fluid sample at 3,000g for 15 min at 4°C to remove cells and debris. *Important: skipping this step may cause filter clogging 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.*
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 A/B/C mixture cannot be stored): 1st add Solution A (0.3 mL); 2nd add Solution B (0.3 mL); 3rd add Solution C (0.3 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 0.9 mL mixture A/B/C to the diluted body fluid sample (from step 3).
7. Cap the 15 mL tube, **gently invert** the tube for at least 10 times to mix well, 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) and** 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.**

Pipet out the Top transparent layer **Remove the Bottom colorless layer** **Keep the Middle fluffy 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" is 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 ExoPure™ 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 ExoPure™ 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.Trouble Shooting:

1. **When there are only 2 layers (top pink/transparent layer and fluffy layer at the bottom) but not 3 layers as expected after step 7 (as shown in the picture at step 8).**
   Pipet out and discard the top pink/transparent layer, then go to step 9 and proceed.
   For some samples, layer separation is not sharp. You can vaguely see 3 layers: 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 30 minutes. Spin the tube at 5,000× g for 3 minutes. Now the mixture appears as 3 layers as shown in step 8. Then Pipet out the Top transparent layer and discard it without disturbing the Middle fluffy layer and proceed to step 9.
2. **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; too much lipid protein in the sample; some precipitate was pipetted in step 15; 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 15.
   - **b.** For some types of samples, the fluff (in step 13 and step 14) is very difficult to be resuspended, and the exosome may be trapped in the fluff. Add the final flow through back to the fluff pellet stored in 4°C (in step 15), 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 types, the production of exosome is low. Increase the initial input medium volume to collect more exosome.

3. **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 bottom layer. Pass the sample through a new column to collect the flowthrough.
4. **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, may not able to lysis exosome to release exosomal protein.
5. **Exosome yield is good, but exosomal RNA level is low.**
   a. RNA degradation. Please check the working environment if it is RNase free.
   b. Also can add spike-in RNA to isolated exosome and then do RNA isolation to control the RNA extraction procedure.
6. **Exosomal RNA yield is good, but cannot get RT-PCR amplification.**
   a. Please check internal control amplification.
   b. Please check the primer sensitivity.

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

FOR RESEARCH USE ONLY! Not to be used on humans.