Exosome Isolation Kit from Breast Milk

Cat#: Exo-BM50

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User Instruction

ExoEZ™
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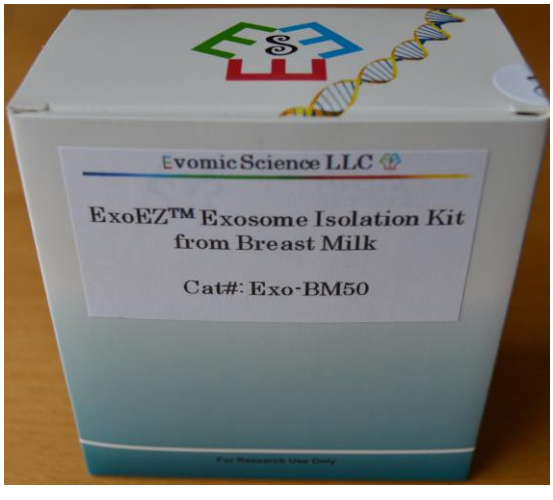

Store kit at +4°C to +8°C on receipt

General Tips for Exosome Isolation

- All biofluids should be considered biohazards and should be disposed according to the researcher's institution, state and federal regulation.
- Personal Protective Equipment should be worn at all the time when working on biofluids.
- Since different biofluids have highly variable compositions, the specifically optimized sample processing for each type of biofluids is required.
- Sample collecting and handling prior to purification can have a significant impact on the purity and yield of isolated exosomes! (Clotilde Théry *et al 2018 Journal of Extracellular Vesicles.*)
- In all processing steps from biofluids, consideration should be taken to prevent lysis of cells. Intracellular vesicles due to cell lysis or platelet activation in plasma case would definitely contaminate your exosome samples. It could result in misleading conclusion.
- If biofluids will not be used immediately, any cells in biofluids must be removed prior to store at -80°C.

Kit Components of Exo-BM50

(Suitable for up to 50 ml of Breast Milk)

Components	Volume
Reagent P1	25 ml
Reagent P2	25 ml
Reagent D	500 µl
Reagent F	500 µl
1xPBS	Not included
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Exosome in Breast Milk

Milk is not only a nutrition source, but also contains biologically important components, cells, and nanoparticles, which have an immune regulatory function. Inappropriate storage of milk at room temperature or colder for more than one hour causes milk cell apoptosis and death, which contaminates the pool of naturally present exosomes. Exosome isolation from milk is further complicated by the abundance presence of fat globules (MFGs), casein micelles, cells and cellular debris. MFG, a lipid droplet covered by proteins and phospholipids, is a kind of vesicle that has largely heterogeneous size and density. Efficient removal of cells and cell debris, casein micelles, and MFGs would significantly improve the quality and yield of the isolated exosomes.

Milk Sample Processing

1. **Freshly collected milk** should be processed within 60 minutes.
2. **Defatted milk**: Fresh milk was centrifuged at 2,000×*g* at 4˚C for 20 min, to remove MFGs, somatic cells, debris, and the cream layer.
3. **Whey**: Defatted milk was pre-warmed for 10 min at 37°C, mixed with 1/100 volume of acetic acid at room temperature for 5 min, and centrifuged at 10,000 x g at 4°C for 10 min to remove milk fat and debris. Casein was pelleted, and the supernatant was filtered with a 0.22 µm membrane and designated whey.
4. Whey can be either immediately processed for exosome isolation or frozen at -80°C until usage.

Exosome Isolation

1. Transfer the desired volume of whey to a new tube and add 0.5 volumes of Reagent P1, 0.5 volumes of Reagent P2, and 1/100th volume of Reagent D and F, respectively. (Refer to the table below for sample volumes)

Supernatants	Reagent P1	Reagent P2	Reagent D	Reagent F
5 ml	2.5 ml	2.5 ml	50 µl	50 µl
10 ml	5 ml	5 ml	100 µl	100 µl

2. Mix supernatants with the exosome isolation reagents well (Do not vortex), and then centrifuge the samples at 2,500g for 15 min at 4°C.
3. After centrifugation, discard supernatants carefully with pipette. Do not touch the soft pellet in the bottom!
4. Transfer the soft pellet with supernatants (200~500 µl) to a 2 ml dolphin microtube and spin down for 3~5 min at 2,500g.
5. Exosomes are concentrated on the interface and bottom phases! Remove the extra reagents / supernatants carefully with pipette! Do not touch the interface and bottom phases!
6. Suspend the concentrated exosomes in 50 µl~300 µl of PBS or your desired buffer.
7. These exosomes are suitable for most of applications, such as RNA isolation, ELISA and western blot, *in vitro* loading of RNAs, and *in vivo* animal study.
8. If purer exosomes (such as for Protein Mass Spectrometer) are desired, exosomes should be further purified by the Exosome Purification kit (Cat# Exo-A300) or immunoaffinity beads, to remove trace contaminated proteins and precipitation reagents.
9. We recommend to use the fresh isolated exosomes immediately. Otherwise please store at 4˚C for overnight, or freeze at -20˚C or -80˚C for longer periods. Note that repeated thaw and freeze cycles can lead to some loss of exosomes.
10. **When exosomes are used for RNA isolation, Do not use classical TRIZOL reagent for miRNA isolation. Using *Quick*-RNA Mini or Microprep Kit from Zymo Research or mirVana miRNA isolation kit from Thermofisher will give a good result, when elution buffer was 95°C RNase-free H_2O.**