Exosome Isolation Kit from Other Biofluids

Cat#: Exo-BF25

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User Instruction

ExoEZ™

Density-based Exosome Isolation Kit
from Other Biofluids:

Cerebrospinal fluid (CSF); Broncho alveolar lavage fluid (BAL);
Saliva; Nasal Secretion; Ascitic Fluid; Amniotic fluid, etc

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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.*

Exosome in Several Biofluids

Cerebrospinal Fluid (CSF)

CSF is the interstitial fluid that bathes the ventricles of the brain, and maintains hydrodynamic pressure, transportation of nutrients, and removal of metabolites from the brain. In general, CSF is clear, colorless, nearly acellular, and contains salt and low concentrations of proteins and lipids. Cells in CSF are fragile to environmental condition changes and will die soon if CSF samples are not processed within one hour. The cell membranes and fragments released from dead cells will greatly contaminate the pool of natural exosomes. Therefore, cells and large vesicles in CSF need to remove prior to sample storage and exosome isolation.

Saliva

Saliva is produced by 3 major paired glands of the sublingual, submandibular, and parotid, and additional smaller glands lining the oral cavity. The different glands secrete various amounts and kinds of exosomes at the different times. It is critically important to document the saliva sampling location and time. Saliva contains cells, membranous vesicles, food debris, glycoproteins, antimicrobial compounds and secreted antibodies etc. Therefore, cells and large vesicles in saliva need to remove prior to sample storage and exosome isolation.

Nasal Secretion

Nasal secretions (NS) are a physical barrier against antigens which are breathed through the nose. Nasal secretion contains cells, membrane vesicles, and proteins. Nasal secretions, in fact, represent the easy and noninvasive samples from the upper airways. Sampling technique, such as, nose blowing, nasal lavage, suction, swabs and spray and absorption techniques, significantly impacts the composition of nasal secretion. It is critically important to document the sampling

techniques of nasal secretions. Therefore, cells and large vesicles in NS need to remove prior to sample storage and exosome isolation.

Bronchoalveolar Lavage Fluid

The alveoli respiratory tract is covered by a thin film fluid secreted by epithelium cells. This particular fluid can be sampled by fiberoptic bronchoscopy technique, as bronchoalveolar lavage (BAL). Saline solutions with low pH and poor nutrients, used for BAL collection, are incapable of sustaining cells. Cells in BAL will die soon if BAL samples are not processed within one hour. Therefore, cells and large vesicles in BAL need to remove prior to sample storage and exosome isolation.

Amniotic Fluid

Amniotic fluid is the fluid that surrounds fetus in uterus. Amniotic fluid is mainly water with salt, proteins, lipids, phospholipids, urea, and cells from the baby. The volume of amniotic fluid is positively correlated with the growth of fetus. Therefore, cells and large vesicles in amniotic fluid need to remove prior to sample storage and exosome isolation.

Biofluid Sample Processing

Most of biofluids could be processed following a similar procedure, as described in the following table. Briefly, samples would be centrifuged at 2000g for 10 min to remove cells and cellular debris etc. Supernatants would be transferred to a new tube and centrifuged at 14,000g for 30 min or be passed through 0.22 μm filter to remove large vesicles etc. After processing, supernatants would be ready for exosome isolation or stored in -80°C for future usage.

Biofluid	1st Centrifuge Speed, Time, Temperature	2nd Centrifuge Speed, Time, Temperature	PBS Dilution
Cerebrospinal fluid (CSF)	2000g, 10min, 4°C	14,000g, 30 min, 4°C	None
Broncho alveolar lavage fluid (BAL)	2000g, 10min, RT	14,000g, 30 min, 4°C	None
Nasal Secretion	2000g, 10min, RT	14,000g, 30 min, 4°C	1:1
Saliva	2000g, 10min, RT	14,000g, 30 min, 4°C	1:1
Amniotic fluid	2000g, 10min, 4°C	14,000g, 30 min, 4°C	1:1
Ascitic Fluid	2000g, 10min, 4°C	14,000g, 30 min, 4°C	1:2

Kit Components of Exo-BF25

(Suitable for up to 25 or 50 ml of Biofluids)

Components	Volume
Reagent P1	25 ml
Reagent P2	25 ml
Reagent D	500 µl
Reagent F	500 µl
1xPBS	Not included
User Instruction	Save Paper Save Life

Exosome Isolation

1. Transfer the desired volume of the above processed biofluids 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.**