Exosome Isolation Kit from Cell Culture Media

Cat#: Exo-CC50

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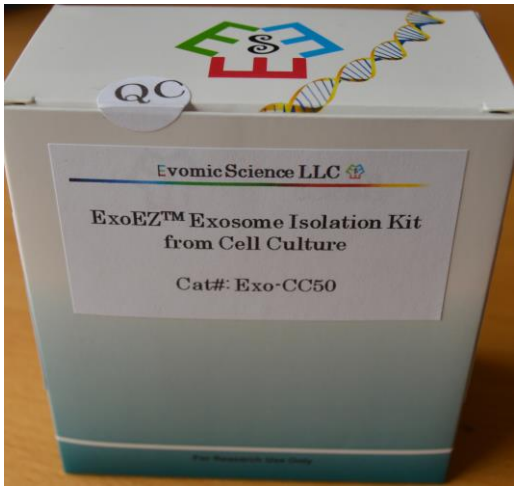
Evomic Science

User Instruction

ExoEZ™

Density-Based Exosome Isolation Kit
from Cell Culture Media
(Suitable for Bacteria, Yeast, Fungi, and Plant and Mammalian)

Cat#: Exo-CC50



Store kit at +4°C to room temperature on receipt

Principle of Exosome Isolation Kit from Cell Culture Media

This product is based on exosome density (1.1~1.21 g/ml) to isolate exosome. Briefly, when cell culture supernatants are mixed with the various density matrix well, the exosome with density (1.10~1.21 b/ml) would be separated from other various density molecules and particles and stay in the bottom phase and its interface after low speed centrifugation (2500g) for 15 min. This method is completely different from the PEG-based water expelled method. The purity and yield using this method is better than using the PEG-based method and ultracentrifuge method. The detailed comparison could be found on website www.evomicscience.com. The following chart outlined the procedures of exosome isolation from cell culture supernatants.

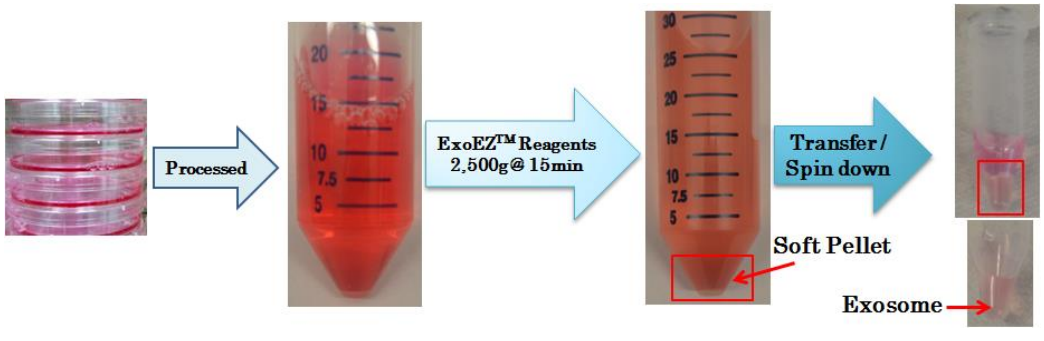


Fig. 1. A brief procedure for exosome isolation from cell culture supernatants. Cell culture supernatants were centrifuged at 2,500g for 10 min and filtered with 0.22 μm or 10,000g centrifugation. The supernatants was transferred to a new tube and mixed with ExoEZ™ exosome isolation reagents. After centrifuged at 2,500g for 15 min, the bottom phase contained exosomes. Transfer this soft pellet to a new microtube and spin down at 2,500g for 3~5 min, remove trace supernatants, the bottom phase was exosome.

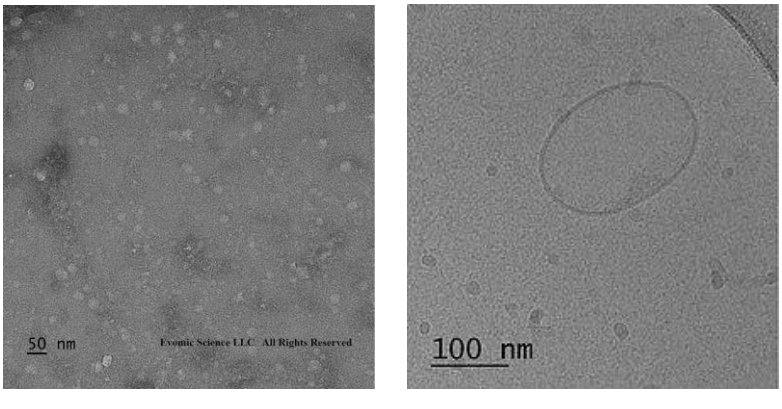


Fig. 2. Electron microscopy of purified exosomes. EM and Cryo-EM images of the exosomes from cell culture supernatants were showed in the left and right of the low panel.

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 Cell Culture Media

Cell culture medium should be collected when the interested cells reach 70-90% confluence. Fresh medium is typically added to cell at 24-48 hours before harvesting supernatant. If any serum will be used in your culture media, make sure that the contaminated exosomes from serum, such as FBS/FCS or others, have been depleted. If you have difficulty obtaining exosome-depleted serum, you can use serum-free or serum-reduced media. Conditioned medium should be stored in either stored at 4°C for days or kept frozen at -80°C for longer periods. Strictly avoid repeated thaw and freeze cycles.

Kit Components of Exo-CC50 (Suitable for up to 50 ml of Cell Culture Media)

Components	Volume
Buffer P1	25 ml
Buffer P2	25 ml
Buffer D	0.5 ml
Buffer F	0.5 ml

Sample Preparation

1. Collect cell culture supernatants.
2. Centrifuge supernatants at 2,500×g for 15 minutes at 4˚C.
3. Carefully transfer supernatant into new tubes and then pass through 0.22 μm filter or centrifuge supernatants at 10,000×g for 10 minutes at 4˚C to remove most of large microvesicles.

Exosome Isolation

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

Processed Media	Buffer P1	Buffer P2	Buffer D	Buffer 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, remove supernatants carefully with pipette. Left 200~300 µl of supernatants in bottom! Do not touch this soft phase in the bottom!
4. Transfer the soft phase with supernatants (200~300 µl) to a 2 ml dolphin microtube.
5. Spin down for 3~5 min at 2,500g.
6. Remove supernatants carefully with pipette! Do not touch the interface and bottom phases!
7. Suspend the concentrated exosomes in 50 µl~300 µl of PBS or your buffer.
8. 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.
9. If purer exosomes (such as for Protein Mass Spectrometer) are desired, exosomes should be further purified by the Exosome Purification Column Kit (**Cat# ExoA300 or ExoB300 or ExoC300**) or immunoaffinity beads, to remove trace contaminated proteins and reagents.
10. 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.
11. **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.**