EV Isolation Reagent FAQs

1. **What samples can I use with the EV isolation reagent?**
   This product can be used to purify EVs from a wide variety of tissue culture media; however, we have not tested it for other biofluids such as plasma, urine, follicular fluid, and breast milk. We expect it to work.
2. **Does the reagent work on plasma or serum samples?**
   This has not been tested by OriGene. Plasma and serum are more heterogeneous types of samples compared to cell culture medium, as it has high levels of proteins and clotting factors, which can be more problematic. Theoretically, the precipitation buffer can precipitate all the exosomes from plasma, but the preparation will likely contain more contaminating proteins. We recommend additional filtration of samples and optimizing incubation conditions to handle plasma and serum samples.
3. **How to keep the cell supernatant before isolation of EVs?**
   When harvesting the cell culture medium, it should be spun down twice to remove cells and debris, as instructed in the product manual. You can keep the cleared medium at 4 degrees for a couple of days or freeze it immediately at -80 degrees for up to months. When processing pre-frozen samples, we highly recommend spinning the "clarified" medium again to remove any cryoprecipitate.
4. **What is the minimal volume of sample I can use for isolation?**
   For cell culture media, we recommend using at least 1 mL of samples in an appropriate tube and at the centrifuge speed specified in the manual.
5. **Can I extend the incubation to overnight before exosome precipitation?**
   Yes, overnight at 4 °C is acceptable without sacrificing quality or yield. Please ensure that the mixture remains upright in the tube without mixing or rocking.
6. **What downstream applications can I use this reagent-isolated EV/exosomes for?**
   The isolated EVs/exosomes by this reagent are intact and fully functional. They can be used for a wide range of downstream applications in functional studies (such as cell-to-cell signaling and vesicular trafficking) and basic biology (including their role in tumorigenesis). They can also be used in biomarker discovery, such as protein and miRNA profiling. Additional clean-up steps are required for exosome proteomics to avoid interference with mass spectrometry analysis.
7. **How can I verify the presence of exosomes?**
   General EV/exosome markers (CD63, CD9, CD81, and Syntenin can be analyzed by Western blotting or ELISA. OriGene provides a variety of antibodies to support EV research. More can be found at www.origene.com. You can also analyze EV particle size and concentration using a nanoparticle analyzer (e.g., NanoSight, ZetaView). EV morphology can be visualized by electron microscopy.
8. **What if there is no pellet visible? How can we ensure that we are not losing the EV/exosome pellet?**
   Cell culture media, like some biofluids, have relatively lower exosomes than plasma or serum samples, and the pellet is faint or invisible in some cases. However, it does not mean a failure in your isolation. We recommend marking the tube so you would know where the EVs will adhere upon spinning, and carefully remove the supernatant completely before resuspension. We don't recommend leaving a significant amount of supernatant, as this can cause excess exosome aggregates to form at the bottom of the tube. Assuming you have not lost the pellet, you can perform NTA or use other tests to confirm your isolation.
9. **What is the best way to resuspend the pellet of exosomes after EV Isolation?**

The EV/exosome is soluble in PBS or many other buffers. We recommend using an exosome-depleted buffer that has been pre-cleared of any particles/aggregates greater than 20 nm. In case of a large pellet from a large volume of samples, you can add resuspension buffer, let it sit for 30 minutes to 1 hour at room temperature, then gently pipette up and down to resuspend the exosomes.

10. **Can I leave some supernatant in the exosome pellets after the centrifugation? Does it interfere with the downstream biological studies?**
    We don't recommend leaving a significant amount of supernatant, as this will cause the exosomes to aggregate at the bottom of the tube. With only trace amounts remaining in the exosome pellet after isolation, it is unlikely to interfere with downstream biological studies. If concerns persist regarding trace amounts of the reagent being present, they can be removed through dialysis or other purification methods.
11. **Can I freeze/thaw my exosomes several times?**
    We also recommend freezing exosomes in single-use aliquots to prevent multiple freeze/thaw cycles
12. **What is the best way to store my EV/exosomes?**
    We highly recommend storing isolated EVs/exosomes in storage buffer at -20 °C or -80 °C for long-term storage. We have found that multiple freeze-thaw cycles can cause damage to the exosomes and reduce their numbers. If multiple applications or thaws will be used for analysis, then aliquot the resuspension into multiple tubes to minimize the freeze/thaw cycle.
13. **How much RNA can be recovered from the exosomes?**
    This can vary depending on the sample type, sample volume, and RNA isolation method. Listed below are some examples: When exosomes are isolated from 10 mL of HeLa cell culture medium using the EV Isolation Reagent, it is possible to recover more than 20 ng total RNA. For exosomes recovered from serum, the exosomal RNA yield can be significantly higher.