**PureExo® Exosome Isolation kit (cell culture media)**

**Cat. #:** P100S (2 reactions); P100 (10 reactions); P100L (40 reactions)

**Trouble Shouting**

1. **The final exosome yield is low.**
   1.1. 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; serum was added in the medium; some precipitation was pipet up in step 14; 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 14.

   1.2. For some type of sample, the fluff (in step 13) is very difficult to be resuspended, and the exosome may be trapped in the fluff. This can be examined by check exosome marker level in step 14 pellet and the final exosome flow-through using ELISA. If the signal from step 14 pellet is high, the exosome release step is incomplete.

      Add the final flow through back to the fluff pellet stored in 4°C (in step 14), pipet up and down **vigorously 60 times**, and shake the tube on a horizontal shaker for **20 minutes**. **Repeat pipetting** up and down vigorously a few times in the middle. Go through another column to collect the exosomes.

   1.3. For some cell type, the production of exosome is low. Generally, the cells produce more exosomes when they are in fast proliferating phase. Tune the cell culture condition (seeding density, splitting intervals etc.) to achieve optimal cell growth condition to collect more exosome. Also increase the initial input medium volume to collect more exosome.

2. **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 **5,000 x g** for **3 minutes**, and carefully pipet out the top and bottom layer. Pass the sample through a new column to collect the flow-through.

3. **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, is not able to lysis exosome to release exosomal protein. We suggest to use our **Exosomal RNA and Protein Extraction Kit**, Cat.#: P200, to extract exosomal protein.

4. **Exosome yield is good, but exosomal RNA level is low.**

   4.1. RNA degradation. Please check the working environment for RNase free. Also can add spike-in RNA to isolated exosome and then do RNA isolation to control the RNA extraction procedure.

   4.2. We suggest to use our P200 kit to extract exosomal RNA.

5. **Exosomal RNA yield is good, but cannot get RT-PCR amplification.**

   5.1. Please check internal control amplification.

   5.2. Please check the primer sensitivity.