**EV sample preparation for isobaric labeling**

Isolate LEVs and SEVs using your method

Keep the pellets in ultracentrifuge tubes at -80C by covering with parafilm

Isolate more LEVs and SEVs and keep them at -80C until enough amount of EVs are collected

Resuspend and lyse frozen EV samples using 60 ul iTRAQ Lysis buffer and combine them in a micro tube for each LEVs or SEVs.

1x iTRAQ lysis buffer: *10 mM HEPES pH 7.5/ 150 mM NaCl/1 or 2% NP-40/0.5% Na-DOC*

Sonicate the lysed EV samples for 15 mins (15 sets of 30 sec high + 30 sec interval) using bioruptor in MCN B0219A. [Note: While sonicating, remove some water using 50ml conical tube every 2-3 cycles and add some ice while in 30 sec interval to keep water cold]

Spin down samples at 15,000 rpm for 30 min at 4oC. Carefully collect supernatant.

Use 2ul of each sample to run on SDS-PAGE gel to confirm approximate equal protein conc between samples.

Use 2ul of each sample to do microBCA for protein concentration

Total 50 ul of sample with 1ug/ul protein is recommended.