

## Vesicle separation from cell supernatant using DynaBead magnetic beads

1. Pellet the cells with appropriate centrifuge conditions (e.g. 300 g, 10')
2. Discard the pellet and put the supernatant to a new tube
3. Spin it down with 2000 g, 20 min
4. Add 15  $\mu$ l beads (previously coated in 1% BSA to 250  $\mu$ l supernatant and incubate it while rotating overnight at 4°C
5. Next day put the samples into Eppendorf tubes and spin it with 300g 5'
6. Discard the supernatant after sipping it with a needle (the magnetic beads will stay at the bottom of the tube, especially if putting the tubes into a magnetic stand)
7. Wash for three times in 150  $\mu$ l AxBB / PBS / NaCl in the magnetic stand
8. After discarding the last washing buffer, sip the beads dry with a needle

Note: you can spin down the beads to the bottom of the tube before sipping it with the needle

9. Resuspend the buffer in the desired amount of buffer and make the analysis it as soon as possible, in the meantime store it at 4°C