Flow Cytometric Analysis of Extracellular Vesicles

Indirect staining on the **surface** of the vesicles

Samples:
isolated large or medium size vesicles from the supernatant of the cell culture. (We always use freshly isolated Evs.)

Preparations:

Buffers:

- Prepare 0,2um filtered PBS or Annexin Binding Buffer.

Anti-body:

- Dilute the antibody ten folds with PBS
- Centrifuge at 12500 g for 1 minute at 4C
- Pipette the supernatant to a new eppendorf tube (**AB**)
- Protect from the light!

Triton-X 100 working solution:

- Dilute Triton-x 100 stock ten folds with d.water
- Filtrate it 0,2um

Sample Preparations:

- add 5-5 uL AB to FACS tubes
- add 10-100 uL EV suspension to FACS tubes. (The volume is depend on the concentration of the EV suspension. Not use too concentrated suspension.)
- add 0,2 um filtered buffer to FACS tubes, such that a final volume can be 300 µL. (Usually this buffer is PBS, but if we use Annexin, we have to use annexin binding buffer)
- Incubation: 15 minutes, 4C, protected from light

- add 5-5 uL secunder AB to FACS tubes
- Incubation: 15 minutes, 4C, protected from light
- Add 50-100 uL count check bead
  (If we have to determine the absolute number of the vesicles, we have to use Count Check beads. For examples: 50uL from the medium count check beads, or 100 uL from the low count check beads, or 50 uL from PKH-beads. Any bead can be used well, but it is always necessary to know how many beads we have put in the tubes, because later we calculate the absolute vesicle number based on this.)

Measuring Extracellular Vesicles by FACSCalibur:

- Open the measuring protocol: Empty/EV protocols/iEV protocol
- Instrument settings: Empty/EV settings/iEV setting
- Acquisition and storage: 1 minute
- After the measurement add 5 uL Triton-X 100 working solution and measure again.

Required Controls:

(These technical controls are required to evaluate the measurement.)

- Unstained sample
- Sample+secunder AB
- Buffer+primer AB+secunder AB

Alternatives:

If we have too big background signal (from the Buffer+ primer AB+ secunder AB control), try to isolate the iEV-s, and add some extra washing steps to the protocol. Work in eppendorf tubes!

- After the first incubation (with the primer AB) add 1 mL filtered PBS and centrifuge it (12 500g, 20 minutes).

- Take off the sup.nat, add 50 uL filtered PBS to eppendorf tube.
- Add 5 uL secunder AB.
- Incubation: 15 minutes, 4 C, protected from light.
- add 1 mL filtered PBS and centrifuge it (12 500g, 20 minutes).
- Take off the sup.nat, add 300 uL filtered PBS to eppendorf tube.
- Pipette the sample to FACS tube.