Flow Cytometric Analysis of Extracellular Vesicles

Small Vesicles measurement with **latex bead**

Samples:
isolated small EVs.
(We use always freshly isolated Evs.)

Preparations:
Buffers:

- Prepare 0,2um filtered PBS or Annexin Binding Buffer.
- Prepare 300nM glycine
- Prepare 0,2 um filtered 10% BSA

Anti-body:

- Dilute the antibody ten folds with PBS (All anti-bodies are diluted, including the isotype.)
- Centrifuge at 12500 g for 1 minute at 4C
- Pipette the supernatant to a new eppendorf tube (**AB**)
- Protect from the light!

Sample Preparations: (bonding with latex beads)

- mix our sEV suspension with beads. We titrated the volume, we need 0,01uL bead (stock) each FACS tube. (So if we have to create 3 different staining combination, we need 0,01 x 3 uL bead. We didn't determine the maximum amount of sEV, because We have found that the more samples the better.)
- Create a "bead control". Same volume bead without samples.
- Incubation: 30 minutes, RT, in the dark
- Add 20 uL of 0,2 um filtered PBS

- Incubation: 30 minutes, RT, pipette up-and-down every 5 minutes gently
- Add 30 uL of 300 nM glycine
- Add 10 uL of 0,2 um filtered 10% BSA
- Incubation: 4C, 1 hour to overnight
- Centrifuge at 2700g for 5 minutes
- Discard the supernatant
- Resuspend beads in 0,2 um filtered PBS. (For example If we have to distribute it for 3 tubes, we suspend it in 3 x 100 uL)
- Add 100-100 uL sample to each eppendorf tube. (We stain the bead control too.)
- add 5-5 uL AB to tubes (to the sample and the bead control too)
- Incubation: 15 minutes, 4C, protect from light
- Add 1 mL filtered PBS to each eppendorf tube.
- Centrifuge at 2700g for 5 minutes
- Resuspend beads in 0,2 um filtered PBS, such that a final volume can be 200 μL.

Measuring Extracellular Vesicles by FACSCalibur:

- Open the measuring protocol: Empty/EV protocols/sEV protocol
- Instrument settings: Empty/EV settings/sEV setting
- Acquisition and storage: 15 000 events
- Triton lysis not works with latex bead

Required Controls:

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

- bead+Unstained sample
- bead+AB (= “bead control”)