Flow Cytometric Analysis of Extracellular Vesicles

Direct staining **inside** of the vesicles

Samples:
Platelet free plasma (PFP)
(Do not use re-freezed samples!)

Preparations:
Buffers:

- Prepare 0,2um filtered PBS and 4%PFA.

Anti-body:

- Dilute the antibody ten folds with PBS (All antibodies 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:

- add 3-3 uL PFP samples to FACS tubes (If looking for a rare population, it is advisable to test isolated iEV samples rather than diluted plasma.)
- add 100-100 uL 0,2 um filtered 4% PFA to FACS tubes.
- add 5-5 uL AB to FACS tubes
- Incubation: 10 minutes, RT, protected from light
- add 0,2 um filtered PBS to FACS tubes, such that a final volume can be 300U uL.
- 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
- Triton lysis not works on fixed samples!

Required Controls:

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

- Unstained sample
- Buffer+AB
- Sample+isotype control