Positive separation of Annexin-labeled mEV-s from blood plasma

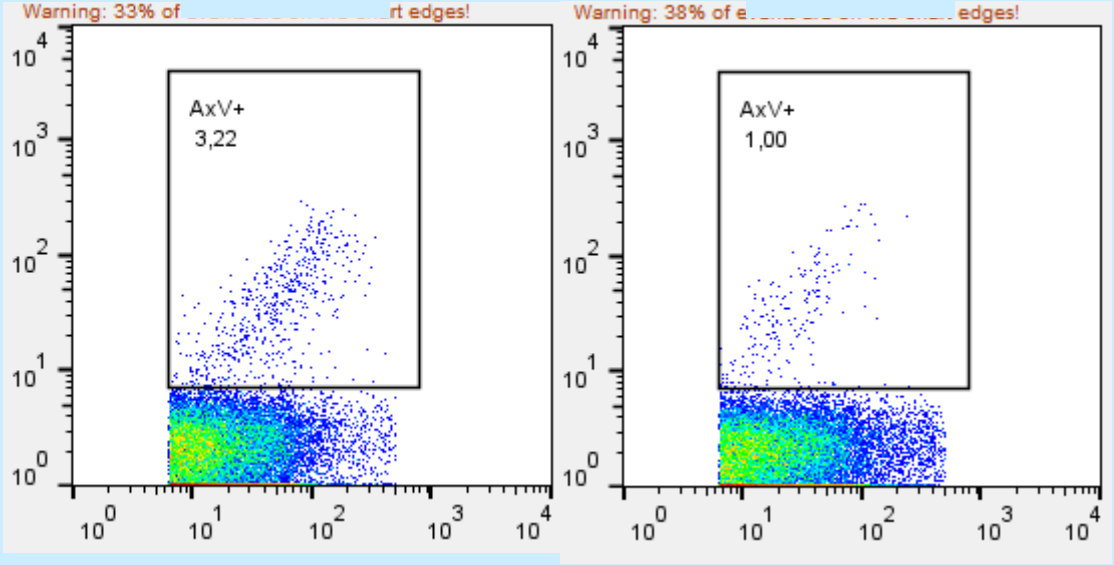
- Preclear medium sized vesicles from PFP (centrifugation, SEC, density gradient centrifugation)
    - avoid using PBS, use HEPES buffered saline instead!
    - optional: wash once in 1,5M NaCl solution to get rid of a part of the protein corona, thus offering a better acces of the PS on the surface)
    - resuspend the pellet in AxBB, or supplement the sample with 2.5 mmol Ca^{2+} so that annexin labeling can be done
- Preconjugate biotinilated Annexin-V with Streptavidin magnetic microbeads in 1:1 ratio at room temperature for at least 15’
- Label the EVs in 100 µl with at least 2 µl of Ax-beads 1^{h} RT while rotating it

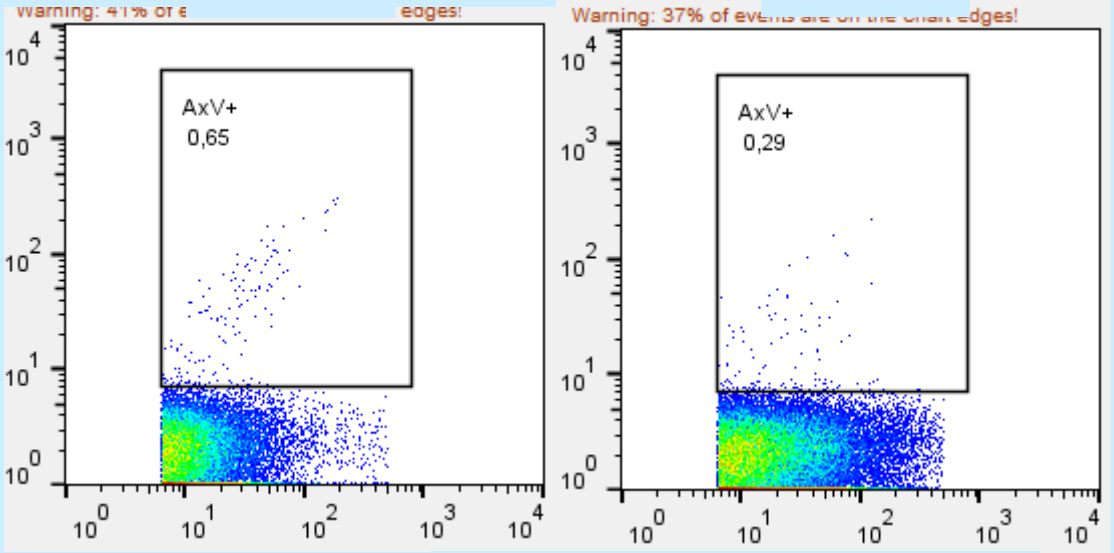
20 ul

2 ul

20 ul

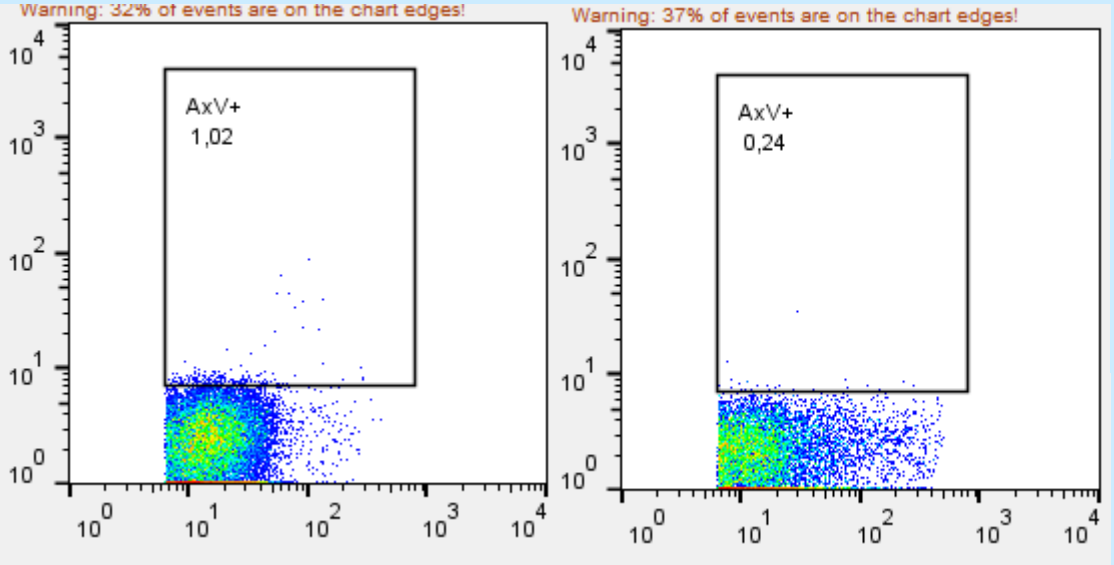
2 ul



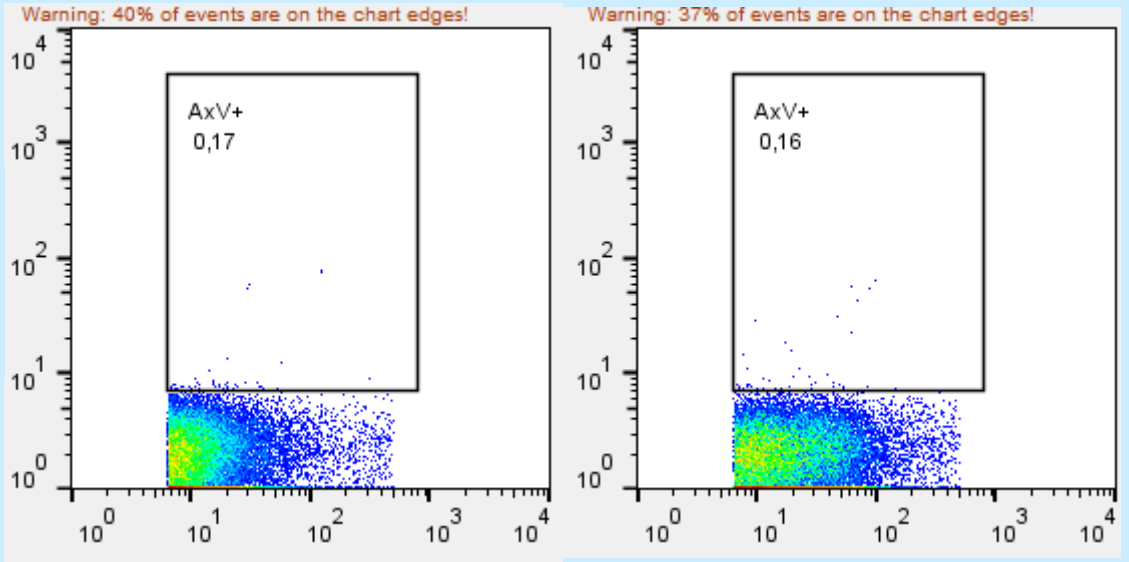


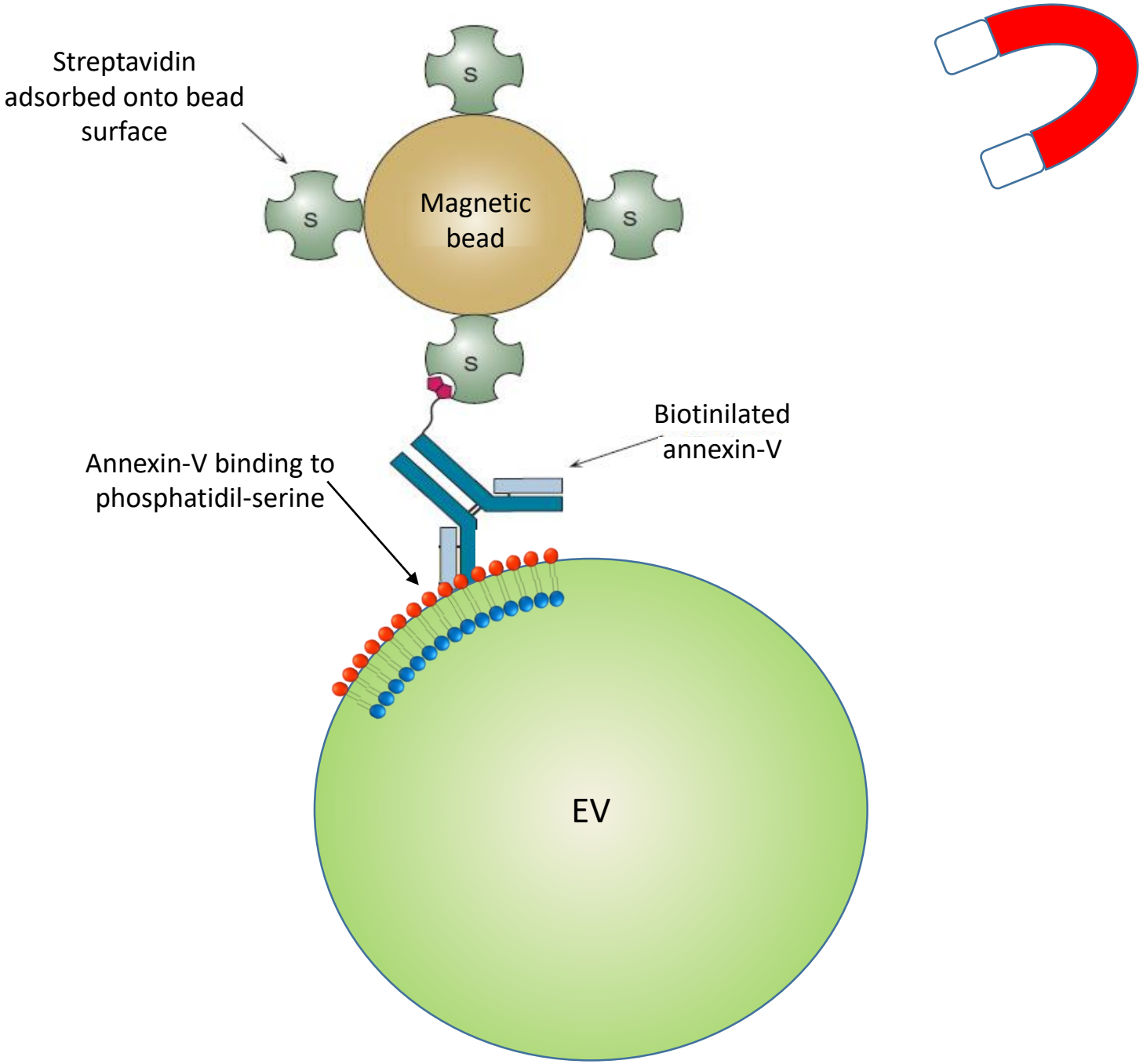
1,5M NaCl

AxBB



+0,1%
TritonX




Streptavidin adsorbed onto bead surface
S
S
S
S
Magnetic bead
Biotinilated annexin-V
Annexin-V binding to phosphatidil-serine
EV

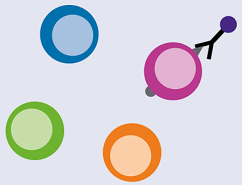
Equilibration Buffer (EB): 1% detergent (Triton-X / Tween / SDS in buffer)

Separation Buffer (SB): the buffer of the sample (NaCl-HEPES)

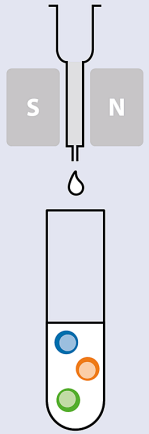
- Attach the column to the magnet stand and place a waste-collector below
- Apply 100 µl of EB on the top of the column. Before moving to the next steps, wait until all the liquid runs through!
- Wash out the detergent with 3-4 x 200 µl SB
- Apply the sample on top of the column
- Wash with 2-3 x 200 µl SB. Retention volume is about 15 µl, but you can collect it with a pipette
- Take out the column from the magnetic field and place it into a 1.5 ml Eppendorf tube
- Add at least 50 µl separation buffer and flush out the magnetically labeled particles by firmly pushing the plunger in the column.

- Clumps bigger that 30 µm will clog the column, as well as bubbles

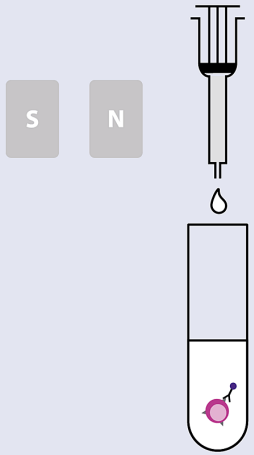
Magnetic labeling



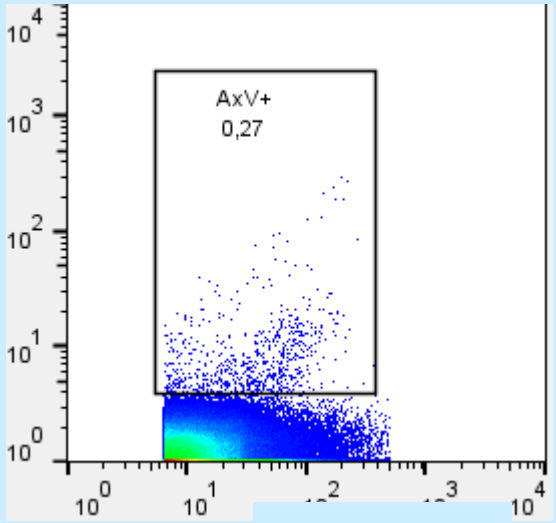
Magnetic separation

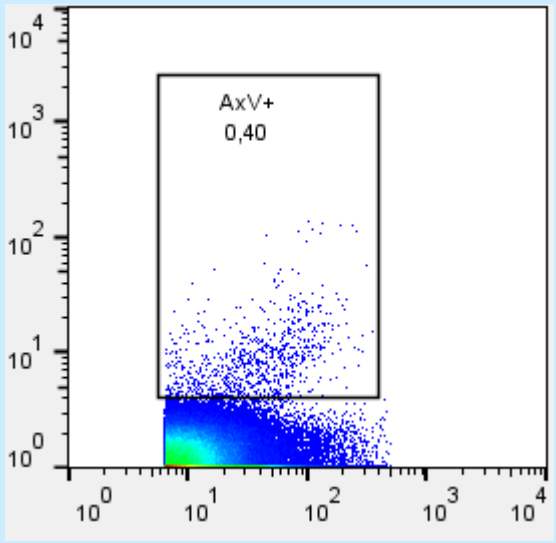


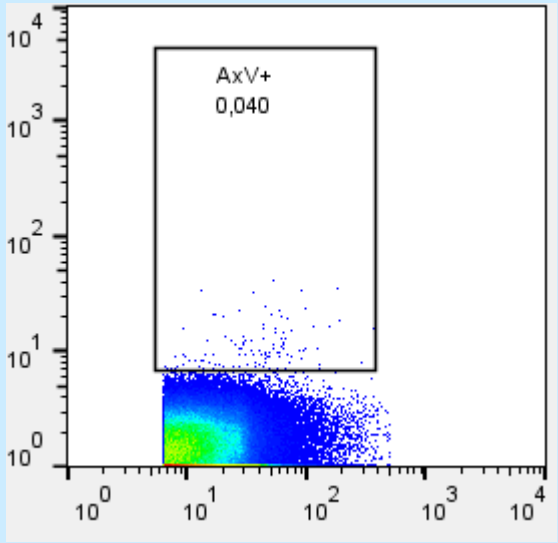
Elution of the labeled cells

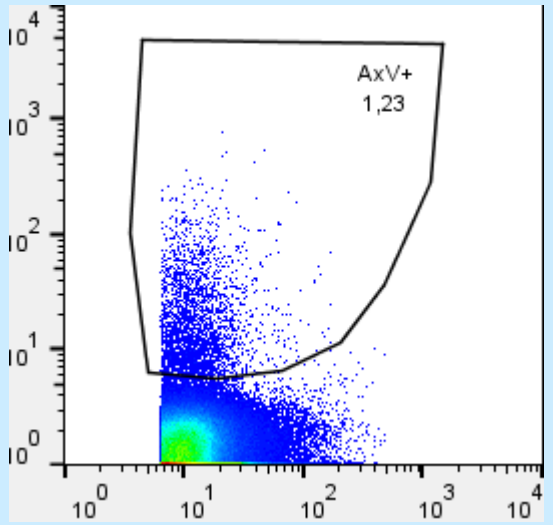


Ax-FITC









20 ul

2 ul

0,5 ul

MV pellet

0,1%
riton-X