Pre-pro-B Sort

- Isolate bone marrow from Bl6 mice
- Resuspend cells and centrifuge **(1500 rpm, 4°C, 1min per 2ml)**
- Discard supernatant
- Resuspend in Ery-Lysis-buffer (2ml/mouse) for 5min at RT
- Stop lysis by adding MACS buffer (2ml/mouse) and centrifuge
- Discard supernatant
- Resuspend in 5 ml MACS buffer and filter (70µm); add 5ml MACS buffer and wash filter; take aliquot and count cells.
- Centrifuge and discard supernatant
- concentrate cells to 10Mio/ml in MACS buffer
- FC block (1:100)
- Centrifuge and discard supernatant
- concentrate cells to 10Mio/ml in **FACS** buffer (without **EDTA**)
- Split Cells:

(Titration: 6 x antibodies x 5 titration steps: 30 vials, each 200.000 cells; total: 6Mio)

- Single staining: 6x 200.000 Cells; total 1.2 Mio; each in 200 µl vol.
- Take remaining cells for panel staining
- Stain with primary and Biotin antibodies for 30min at 4°C.
- Centrifuge and add **FACS buffer** 1ml/10Mio Cells
- Stain with secondary antibody (SA) for 30min at 4°C.
- Centrifuge and add MACS buffer 1ml/10Mio Cells

Material:

Ery-Lysis-Buffer
0,15 M NH4Cl
0,02 M HEPES
0,1 mM EDTA

MACS Buffer
1x PBS
2 mM EDTA
2% FCS

FACS Buffer
1x PBS
2% FCS
Cell strainer 70µm Nylon (BD Falcon #352350)

Panel staining:

Primary staining:	**Secondary staining:**
AA4.1 (CD93)-FITC	Streptavidin A350
cKIT-PE	
B220-PerCP	
HSA (CD24)-PE-Cy7	
CD43-APC	
CD19-APC-Cy7	
Cd11b Biotin (dump channel)	
CD3 Biotin (dump channel)	
Ly6G Biotin (dump channel)	

Staining of prepro B Cells:

AA4.1 POS
CKIT POS
B220 POS
HSA Medium
CD43 POS
CD19 NEG
Dump channel NEG