BM Staining

- Isolate bone marrow from Bl6 mice:

1.) collect all legs needed for the experiment
2.) flush the bones with ice-cold MACS buffer into a 6 Well Plate (filled with 2ml MACS buffer; ice-cold)

- Filter Cells with a 70µm cell strainer; add additional MACS buffer and wash filter

- Centrifuge **(1500 rpm, 4°C, 10 min)**

- *Do Ery-Lysis only if necessary*

- *As some of the antibody-stainings are Ca2+ dependent EDTA could disturb your staining.*

If your staining panel works with EDTA, MACS buffer is recommended from this step on.

- Discard supernatant and resuspend in 1 ml **MACS/FACS** buffer (+ - **EDTA**)

- count cells

- concentrate cells to a maximum of 50Mio/ml in **MACS/FACS** buffer (+ - **EDTA**)

- Stain with primary antibodies for 45min at 4°C.

- Centrifuge and add **MACS buffer** (with EDTA) 1ml/30Mio Cells

Material:

Ery-Lysis-Buffer
0,15 M NH_4Cl
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)