**Isolation of Naïve, non-activated T cells by negative selection**

Remove spleen into T cell medium (sterile, 4C TC room), tease into cell-single suspensions with frosted slides and filter through a 70m cell strainer (bottom drawer cabinet).

Spin 5‘ @1300. Aspirate sup and flick the pellet.

Add 1ml RBC Lysing Buffer (Sigma R7757, shelf TC room, sterile) and lyse for 1.5‘ @room temp.

Flood with 1x PBS. Spin 5‘ @1300. Resuspend in MACS buffer:

150ul per 1 spleen/250ul per 2 spleens/350ul per 3 spleens/400ul per 4 spleens

and add:

for **CD4+ CD25-**T cell isolation: negative selection coctail containing biotin-conjugated mAbs (anti- CD8, CD11b, CD11c, CD25, CD45R-B220, CD95-NK1.1, TER119) [1:200]f

for **CD4+ CD8+ CD25-**T cell isolation: negative selection coctail containing biotin-conjugated mAbs (anti- CD11b, CD11c, CD25, CD45R-B220, CD95-NK1.1, TER119) [1:200]f

for **CD8+ CD25-**T cell isolation: negative selection coctail containing biotin-conjugated mAbs (anti- CD4, CD11b, CD11c, CD25, CD45R-B220, CD95-NK1.1, TER119).

7ul per 1 spleen/10ul per 2 spleens/12ul per 3 spleens/15ul per 4 spleens

To make the negative selection coctail, mix 50 ul of each of the biotin-conjugated mAbs for a total of 350ul.

\* All biotinylated Abs are in 4C TC room fridge in „Biotin Abs“ box

Incubate 20‘ at 40C. Wash with 10ml MACS buffer.

Spin 5‘ @1300. Resuspend in half volume of MACS buffer + 20ul biotin-binding beads per spleen and 5-10ul per MLN (a-Biotin Microbeads 120-000-900 Miltenyi Biotech, 4C TC room fridge, sterile).

Incubate 15‘ @40C. Complete to 1ml MACS buffer and apply to column (column has to be primed with 1ml MACS).

Collect flow through into 15ml Falcon tube containing 2-4ml T cell medium.

Wash column 2x w/1ml and 2x w/3ml MACS buffer. Spin 5‘ @1300.

Resuspend in 2ml T cell medium and count cells.

**Don’t forget to wash the column after you are done for reuse purposes (protocol in TC room).**

**Each column is $15!!!!!**

**Write date, how many times used and your initials. How well it works next time depends on how well u washed it.**