

Intracellular pAkt Stain

This protocol is for stimulating NK cells, but can be adapted to your favorite cell type.

Spin down 2×10^6 cells in six separate 15 mL tubes for each mouse

Resuspend in 10 uL Fc Block. 5' RT

Add 1 uL anti-NK 1.1 Biotin to all EXCEPT UNSTIM samples. 15' on ice. (Meanwhile, prepare 20 uM SA)

Wash with 500 uL cold PBS. Spin down.

Keep pellets on ice until stimulation. To stimulate, add 100 uL of 20 uM SA in PBS and incubate in 37 C water bath for designated time.

To stop stimulation, add 65 uL of 10% Formaldehyde. Vortex. Incubate at RT 15'

Add 1 mL 0.12% Triton. Vortex briefly. RT 30'

Add 1 mL Incubation Buffer (0.5% BSA/PBS) to wash.

Resuspend in 500 uL of cold 50% MeOH. Incubate at RT 10'

Wash 2x with 1 mL Incubation buffer.

Resuspend in primary Ab 60' (Cell Signaling #2965 1:100), dark, RT

Wash with 1 mL Incubation Buffer.

Resuspend in 100 uL secondary Ab (Cell Signaling #4412S 1:1000) 30' RT dark.

Wash with 1mL incubation buffer. Resuspend in 300 uL Incubation buffer to run on flow cytometer