Extracellular (surface) staining

- 2x wash cells with 200µl PBS (500-600g, 2min., 4°C).
- In case of purified antibodies:
    - Block with serum (same species as stained cells): 100µl per well, 10min., 4ºC
    - 2x wash with 200µl PBS, add 10µl of purified antibody and incubate 30min., 4ºC
    - 2x wash with 200µl PBS, block with serum (same species as used to create the secondary antibody): 100µl per well, 10 min., 4°C.
    - 2x wash with 200µl PBS, add secondary antibody (stained) and incubate 25min., 4ºC, in dark.
    - 2x wash with 200µl PBS and continue with biotinylated and stained antibodies.
- Add 10µl primary antibodies (either stained or biotinylated, single or mixes) and incubate 30min., 4ºC, in dark.
- 2x wash with 200µl PBS.
- Add 10µl secondary antibodies (conjugated streptavidin) in case biotinylated antibodies were used and incubate 25min., 4ºC, in dark, then 2x wash with 200µl PBS.
- In case of further intracellular staining, see protocol Intracellular staining.
- Resuspend cells in desired volume of PBS (50-100µl) and if needed, transfer to microtubes (if not using LSRII – HTS).