Intracellular/Extracellular localisation of receptors by flow cytometry

Please note – this protocol has been optimised for certain conditions and is meant as a guide only, you may need to alter some parameters to suit your own experiment

1. Centrifuge cells and resuspend to approx 4 x 10^6 viable cells/ml in PBS + 4% paraformaldehyde
2. Incubate on ice for 45 minutes to fix
3. Take an aliquot of cells off (approx half, or whatever is required) and transfer to a new tube ('Tube #2'). These will not be permeabilised.
4. Wash both tubes in staining buffer, and resuspend to original volume in:
5. Tube #1: saponin buffer (staining buffer + 0.1% saponin)
6. Tube #2: staining buffer
7. Incubate for 12 minutes on ice, mixing half way through incubation
8. Tube #1 ONLY: wash with staining buffer, and resuspend to original volume in staining buffer. Then add a 1/5 volume of saponin buffer, to maintain complete permeabilisation.
9. Proceed with usual staining protocol, but maintain Tube #1 cells in staining buffer containing 1/5 saponin buffer (eg, add 10 μl saponin buffer to the ~50 μl of cell suspension after each wash)

Note: fixation is essential to the procedure, but may damage some epitopes due to extensive cross-linking, resulting in poor or absent antibody binding. The longer the gap between fixation and addition of antibody, the more likely this is to occur, as cross-linking continues even after removal of the paraformaldehyde.