

General surface staining protocol:

Adherent cells should be trypsinized, or otherwise chemically/mechanically, removed from the tissue or plate.

Cells should be stained in PBS + BSA or FBS (2%) in small volumes.

1X10⁶ cells in 100 ul

20 minutes with a primary antibody

Wash once with PBS

10-20 minutes with the secondary antibody

Can be done on ice or at room temp

Should be done in reduced light as fluorochromes are light sensitive

Wash once following secondary antibody

Fix and store cell in 4% paraformaldehyde @ 4 degrees

If doing intracellular staining, permabilize cells following trypsinization and surface staining then stain for intracellular proteins.

For CFSE labeled cells, harvest, wash and fix.