

Intracellular Antibody Staining

Unfortunately there is no one "right" protocol. A good place to start is cytofix cytoperm from BD. Or you could chose to optimize a protocol from a reference for your particular cell type. A variety of detergents can be used (NP-40, Triton-X, SDS, Saponin) as well as a number of cell permeabilization chemicals (EtOH, Formaldehyde, Paraformaldehyde, Gluteraldehyde).

Additional consideration of the molecule to be stained is important. Secretion of molecules such as interferons, chemokines, and cytokines can be blocked by monensin and brefeldin, commercially available as golgi plug and golgi stop.

Intracellular staining should follow staining of surface molecules and be concurrent or proceed nuclear staining.

Secondary antibodies are large proteins which diffuse into cells and "get trapped" in cell pellets, cell membranes, etc. The antigen or Ig class specific (the Fc portion) of antibodies are commercially available labeled with a number of fluorochromes for in flow and microscopy applications; these have less background staining in part because they are smaller molecules and more easily washed from cells.