Suggestions or questions to:
Rebecca Williams, Director
Cornell Imaging, BRC
rw36@cornell.edu

Immunostaining protocol for attached cells.

Phosphate Buffered Saline (PBS):
Formaldehyde: 16%, methanol free, Polysciences, Inc. (cat# 18814). Use fresh, store opened vials at 4°C in dark and dilute in PBS for use.
0.2% Triton X-100 in PBS
1% BSA in PBS (BSA-PBS)

1) Fix cells with 4% formaldehyde for 15 min.
2) Rinse several times in PBS.
3) Permeabilize cells in 0.2% Triton X-100 for 5 minutes.
3) You may want to do a blocking step in BSA-PBS for 15 min to reduce non-specific sticking of the ab. However recent literature suggests that this may be unnecessary and even anti-productive (See Scientific Reports, 2011, 1:28)
4) Dilute your primary Ab in BSA-PBS. Use manufacturer recommendations (usually to a final concentration of 1-5ug/ml). Incubate with cells for 30 min.
5) Rinse several times in PBS.
6) If your primary antibody was unlabeled repeat #4 with a secondary Ab. Use of secondary antibodies amplifies your signal, but also your non-specific staining.

Notes about antibodies: The dogma is that neither primary nor secondary Ab should be from the same species as the cells you are staining or from the same species as each other. It is also preferable to have Ab's have been pre-absorbed against the same species as the cell species -- but these are hard to find.