Immunocytochemistry Protocol

This can be performed either on plated cells, or on arteries pinned out on sylgard-coated 6 well dishes.

Time	**Procedure**	**Treatment**	**Purpose**
2x10 min	Fixation	Paraformaldehyde (4%) in PBS	Cross-link cell proteins
3x rinse; 1x 5 min	Quenching	Glycine (0.1M) in H_20	Deplete excess fixative
3x rinse	Rinse	PBS	Wash away glycine
10 min	Permeabilization	Triton-X100 (0.2%) in PBS	Open holes in cell membrane
3x rinse	Rinse	PBS	Wash away Triton-X
3x rinse	Rinse	Antibody wash solution	Wash away PBS
1 hour	Block	5% BSA	Block non-specific sites
3x rinse	Rinse	Antibody wash solution	Remove excess blocking solution
2 hours (or O/N in fridge)	Primary antibody	1o antibody in buffer	Localize protein of interest
3x rinse	Rinse	Antibody wash solution	Remove excess antibody
1 hour	Secondary antibody	2o antibody in buffer	Label 1o antibody with fluorescent probe
3x rinse	Rinse	Antibody wash solution	Remove excess antibody
For cells:			
20 min	Mounting	Anti free radical treatment slide, coverslip seal	Preparation for microscopy