IMMUNOLOCALIZATION (FLUORESCEIN)

1. Dewax slides 2 x 10' in histoclear.

2. Rehydrate by passing through the following ethanol series (2' each):
   99% ethanol
   99% ethanol
   95% ethanol
   85% ethanol
   70% ethanol
   50% ethanol
   30% ethanol
   dH_2O
   dH_2O

3. Incubate in **PBS/BSA** for 5' at room temperature.

4. Drain slides, place in a large petri dish containing wet tissues around the side. Put 50-100 µl 0.1% Goat IgG in **PBS/BSA** on each slide. Cover petri dish and incubate for 15' at room temperature.

5. Drain slides and rinse in **PBS/BSA**.

6. Drain slides, place back in petri dish and put 50-100 µl 1° antibody on each (1/10 – 1/100 dilution in **PBS/BSA**). Incubate for 15' at room temperature.

7. Wash 2 x 15' in **PBS/BSA**.

8. Drain slides, place back in petri dish and put 50-100 µl FITC conjugated
   2° antibody on each (1/10 – 1/100 dilution in **PBS/BSA**). Incubate for 15' at room temperature.

9. Wash 2 x 15' in **PBS/BSA**.
10. Stain with 100 µM Evans Blue in **PBS/BSA** for 10' at room temperature. (optional).

11. Rinse in **PBS/BSA**.

12. Mount in 2:1 glycerol:PBS containing 2% N-propyl galate. Seal coverslips with nail varnish. View under fluorescent microscope with FITC filter.

PBS/BSA (1L)

0.15M NaCl	30 ml 5 M
10mM phosphate buffer pH 7.0	10 ml 1 M
1mgml^{-1} BSA	2 g