

### **Erithryna cristagalli (EC lectin) staining:**

- 1- Bake the slides 2hs to ON @ 40°C.
- 2- Wash 2x 10 min in TBS (50 mM Tris-HCl pH=7,6; 150mM NaCl)
- 3- Block 2hs with 10% FBS in TNT (50 mM Tris-HCl pH=7,6; 500mM NaCl, 0,5% Triton)
- 4- Incubate with FITC-EC lectin (1/1000, Vector Labs, #FL-1141) in 2% FBS in TNT for 1h @ RT.
- 5- Wash 3x 10 min in TBS
- 6- Mount with Mowiol.

### **Double Immuno staining with Erithryna cristagalli (EC lectin):**

- 1- Bake the slides 2hs to ON @ 40°C.
- 2- Wash 2x 10 min in TBS (50 mM Tris-HCl pH=7,6; 150mM NaCl)
- 3- Block 2hs with 4% Normal serum in TNT (50 mM Tris-HCl pH=7,6; 500mM NaCl, 0,5% Triton)
- 4- Incubate with primary antibody O.N. @ 4°C in 4% normal serum in TNT. i.e. anti-Gi2 $\alpha$ a (1/300) or anti-Gio (1/200) and OMP (1/3000) or biotinylated-*Bandeirae Simplifolia* lectin (1/1000, Sigma, #L-2140).
- 5- Wash 2x 10 min in TBS
- 6- Incubate FITC-EC lectin (1/1000, Vector Labs, #FL-1141) or secondary antibody in 4% normal serum in TNT for 1hr. @ RT (i.e. or FITC-donkey anti rabbit (1/50) and Cy3-donkey anti goat (1/500) or Cy-3 Streptavidin (1/500))
- 7- Wash 3x 10 min in TBS
- 8- Mount with Mowiol.