**Antibody Protocol for 40-50 Micron Thin Tissue Sections with EYFP and c-Fos**

**Day 1**

Volume 1 ml/well

1. 3x washes in PBS for 10 min each at RT.
2. Block in 10% NDS (in PBST: 0.5% Triton X-100 in 1x PBS) for 2 hr at RT.
3. Incubate in primary antibody in PBST overnight at 4°C.

Use:

* 1. Anti c-Fos Rat monoclonal (SySy 226 017, 1:5000)
  2. Anti GFP Chicken polyclonal 10mg/ml (Abcam ab13970, 1:2000)

**Day 2**

1. 3x washes in PBS for 10 min each at RT.
2. Incubate in secondary antibody in PBS for 2 hr at RT.

Use:

* 1. Alexa 647 conjugated Donkey Anti Rat IgG (Abcam 150155, 1:500)
  2. Cy2 conjugated Donkey Anti Chicken IgG (Jackson ImmunoResearch 703-225-155, 0.5 mg, 1:250). Rehydrated with 0.4 ml dH20 and 0.4 ml Glycerol to a concentration of 0.625 mg/ml.

1. 3x washes in PBS for 10 min each at RT.

Optional:

* 1. 1x wash of 1:1,000 Hoechst in PBS for 10 min at RT.
  2. 3x washes in PBS for 10 min each at RT.

1. Mount slides. Lay flat to dry for approximately 20 min (light protect with foil).
2. Add a drop of Prolong Gold antifade reagent (Invitrogen, P36930) or Fluoromount G. Coverslip and seal with clear nail polish; allow to dry and store at – 20C.