

Antibody Protocol for 40-50 Micron Thin Tissue Sections with EYFP and c-Fos
Denny Lab
2/1/22 MS

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:
 - a. Anti c-Fos Rat monoclonal (SySy 226 017, 1:5000)
 - b. 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:
 - a. Alexa 647 conjugated Donkey Anti Rat IgG (Abcam 150155, 1:500)
 - b. Cy2 conjugated Donkey Anti Chicken IgG (Jackson ImmunoResearch 703-225-155, 0.5 mg, 1:250). Rehydrated with 0.4 ml dH2O and 0.4 ml Glycerol to a concentration of 0.625 mg/ml.

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

Optional:

- a. 1x wash of 1:1,000 Hoechst in PBS for 10 min at RT.
 - b. 3x washes in PBS for 10 min each at RT.
4. Mount slides. Lay flat to dry for approximately 20 min (light protect with foil).
 5. 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.