

**iDISCO-based Antibody Protocol for 100 Micron Thin Tissue Sections with c-Fos
(SySy)**

**Denny Lab
08.11.21 (MS)
1.3.2021 (modification MJ)**

Cut whole brain into 100-micron thin tissue sections (about 100) and put sections in a 12 well plate. Each well should be filled with PBS and have about 8-9 sections.

Day 1-3

Volume 1 ml/well

1. Wash in 1X PBS 3 times for 10 min each
2. Dehydrate in 50% methanol/PBS at RT for 2.5 hr.
3. Wash in 0.2% PBST (1X PBS/ 0.2% Tween) 3 times for 10 min each.
4. Block in 0.2% PBST/ 10% DMSO/ 6% NDS for 2 hr at RT.
5. Wash in PTwH (1X PBS/ 0.2% Tween-20 with 10ug/ml heparin) 3 times for 10 min each at RT
6. Place in primary antibody in PTwH/ 5%DMSO/ 3%NDS for 3 days at 4C.

Use:

Anti c-Fos Rat polyclonal (SySy 226 017, 1:5000)

If GFP labeling is also needed add:

Previously: Anti GFP Chicken polyclonal IgY (Aves, GFP-1020, Lot 0511FP12, 10 mg/ml)

**Currently: Anti GFP Chicken polyclonal 10mg/ml (Abcam ab13970, 1:2000)
(used to do 1:500 but had high background)**

Day 4-5

1. Wash in PTwH (1X PBS/ 0.2% Tween-20 with 10ug/ml heparin) 3 times for 10 min each at RT
2. Place in secondary antibody in PTwH/ 3%NDS for ON at 4C.

Use:

Alexa 647 conjugated Donkey Anti Rat IgG (abcam ab150155, 1:500)

If GFP labeling is also needed add:

Cy2 conjugated Donkey Anti Chicken IgG (1:250). Rehydrated with 0.4 ml dH2O and 0.4 ml Glycerol to a concentration of 0.625 mg/ml.

3. Wash in PTwH (1X PBS/ 0.2% Tween-20 with 10ug/ml heparin) 3 times for 10 min each at RT
4. Optional: Add hoecht (1:10,000) in PBS for 15 min.
5. Wash in 1X PBS 3 times for 10 min each at RT
6. Mount slides. Lay flat to dry for approximately 40 min (light protect with foil).
7. 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.

Day 1 - Thursday	6 hr + ...
Day 2 – Fri	...
Day 3 – Sat	...
Day 4 – Sunday	... + 0.5 + ...
Day 5 - Monday	3

(2.15.2018: anti-GFP antibody and mounting medium updated. After mounting the slides, it may be enough to wait just 20 min before coverslipping)