**Immunofluorescence Staining Protocol (Anti-HA antibody)**

Rob Sears 11.26.18

**Cryosection procedure:**

1. Perfuse animals with ice cold or RT 4%PFA (temp does not seem to matter)

2. Fix the brain with 4% PFA @ 4°C O/N.

3. Transfer brain sample to 30% sucrose/1xPBS (0.01M PBS), allow sample sink @ 4°C for about 48hr. If brain doesn’t fully sink, it’s OK.

4. Section on freezing microtome and store tissue in 0.01M PBS+ 0.01% NaAz @ 4°C until ready for IHC

**Staining procedure:**

5. Block tissue in 1% BSA in 1X PBS for 30 minutes to 1 hour, rocking\* @ RT

6. Incubate the sections with primary antibody (1:5,000, Calbiochem Rabbit anti-c-Fos (ab5) and Abcam chicken anti-GFP 1:2,000 (ab13970) in 1% BSA + 0.2% Triton X-100 rocking @ RT O/N.

7. Wash the section with 1X PBS three times @ RT, 5 min/each.

8. Incubate the sections with secondary antibody (1:200) in 1X PBS rocking @ RT for 30’ to 1 hour.

9. Wash the sections with 1X PBS three times at RT, 5 min/each.

10. Mount the sections on subbed (or frosted) slides, let dry just long enough so that the sections don’t float/move on the slide.

11. Wipe off excess buffer with a kimwipe OR lay the slide flat and add a few drops of PBS\*\*

12. Add 3-4 drops of aqueous mounting medium and coverslip (I prefer Prolong Gold from Invitrogen).

\*I use scintillation vials on their sides for all rocking steps.

#Some use 1:250 for anti-HA, which seems to work better in some cases

\*\*The PBS method may preserve signal

**Antibody:**

**Primary antibodie:** Cell signaling, HA-Tag (C29F4) Rabbit mAb, Cat # 3724

**Secondary antibody:** Invitrogen, Alexa Fluor® 488 goat anti-rabbit IgG (H+L), Cat# A-11008