**Chromagen (VIP) Immunostaining Protocol**

Modified from Bryan Roth’s protocol (DREADD Wiki) by Rob Sears 2\_26\_14

**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. Unless cutting on a vibratome, transfer brain sample to 30% sucrose/1xPBS (0.01M PBS), allow sample sink @ 4°C for about 48hr. If brain doesn’t 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. Incubate tissue in 1% H202 for 1 hour

6. Block tissue in 1% BSA +0.2% Triton in 1X PBS for 30 minutes to 1 hour, rocking\* @ RT

7. Incubate the sections with primary antibody (1:500 for R anti-HA, 1:1000 R anti-GFP or 1:500 R anti-dsRED, 1:10,000 anti-cFos) in 1% BSA + 0.2% Triton X-100 rocking @ RT O/N.

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

9. Prepare ABC: 2 drops A, 2 drops B per 5 mL 1X PBS and let incubate at RT

10. Incubate the sections with secondary antibody (Biotinylated anti-Rabbit IgG, 1:200) in 1% BSA (in 0.01M PBS) rocking @ RT.

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

12. Incubate tissue in ABC solution for 30 min

13. Prepare VIP: 3 drops of 1, 2, and 3 and H2O2 per 5 mL 1X PBS. Mix after adding each reagent.

14. Incubate tissue in VIP until dark enough to see staining and transfer to 1X PBS.

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

16. Mount the sections on subbed, let them dry for at least an hour in a dark place

17. Dehydrate tissue using standard protocol

18. Add 3-4 drops of mounting medium (e.g. DPX or Permount) and coverslip

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

**Antibody:**

**Primary antibodies:**

1.Cell signaling, HA-Tag (C29F4) Rabbit mAb, Cat # 3724

2. Invitrogen, GFP polyclonal Rabbit, Cat # A-11122

3. Clontech, dsRed polyclonal Rabbit, Cat # 632496 (for mCherry)

4. EMD/Millipore/Calbiochem rabbit anti c-Fos (ab5)

**Secondary antibody:**

**Biotinylated Goat Anti-Rabbit IgG Antibody**

**Other reagents:**

**1. VECTASTAIN Elite ABC Kit (Standard), PK-6100**

**2. VECTOR VIP Peroxidase Substrate Kit, SK-4600**