**Hippocampal Immunocytochemistry Protocol**

from Shernaz Bamji

**Preparation:**

1. Antibody calculations (diluted in ~120µl of 1% goat serum/PBS per coverslip);
2. Pre-warm 4% PFA/4% sucrose solution and goat serum to 37°c in a water bath;
3. Dilute goat serum with 1X PBS to 1% and 10% solutions.

**Day 1:**

1. Remove all medium from each well;
2. Add 4% PFA/4% sucrose solution slowly with P1000 pipette (0.5ml/well);
3. Incubate for exactly 10 minutes at room temperature;
4. Slowly aspirate PFA/sucrose mixture and then wash with 1X PBS at 1ml/well (or mount them);
5. Add 0.1% Triton-X (in PBS) at 0.5ml/well;
6. Incubate for exactly 10 minutes at room temperature;
7. Slowly aspirate Triton-X solution and then wash with 1X PBS at 1ml/well;
8. Add 0.5ml of 10% goat serum (in PBS) to each well;
9. Cover well plate and leave for 1 hour at room temperature. In the meantime, ~10 minutes before finishing, make up 1°AB solutions and place on ice;
10. Cover the well plate lid in parafilm, remove coverslips from wells, place them on the parafilm and add 110-115µl of the primary antibody solutions to corresponding coverslips;
11. Leave overnight at 4°c.

**Day 2:**

1. Place coverslips back into the wells with 1ml/well 1X PBS;
2. Wash three times with 1ml/well of 1X PBS (storing plates in the dark for 10 minutes between each wash) - washing is critical! In the meantime, make up 2°AB solutions;
3. Remove coverslips from wells, place them on the parafilm and add 110-115µl of the secondary antibody solutions to corresponding coverslips;
4. Cover and leave for 1 hour at room temperature in a humidity chamber to prevent evaporation;
5. Wash the wells three times with 1ml of 1X PBS (storing plates in the dark for 10 minutes between each wash) - washing is critical!
6. Mount onto labelled slides using around ~8µl or one drop of Molecular Probes ProLong Anti-fade mounting media. If using a pipette, take up 10µl to prevent creating bubbles;
7. Place in flat slide box at 4°c overnight to dry.

**Day 3:**

1. Add nail polish to seal the next day.