



Cornell Heart Lung Blood Resource for Optogenetic Mouse Signaling
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Anti-GFP IHC of paraffin embedded sections for detection of GCaMP

1. Deparaffinize the sections
2. Wash the slides in DI H₂O, 3X.
3. Retrieve antigen by steam treatment in a vegetable steamer.
 - a. Fill steamer with water to the top of the tray and preheat for 5' at high setting. Include jars with blank slides as necessary.
 - b. Fill a coplin jar with 10 mM citrate buffer (pH 6.0) and place slides in the slots. Screw the lid loosely.
 - c. Distribute the jars evenly in the steamer and heat for 5' at 100% power setting.
 - d. Remove steamer and check buffer level. Refill if necessary and reheat for 15' at 30% power setting.
4. Remove the steamer from the microwave and cool to ROOM TEMPERATURE after adding cold water into the steamer. DO NOT REMOVE LID FROM COPLIN JAR CONTAINING THE SECTIONS.
5. Rinse the slides in DI H₂O.
6. For HRP detection, pretreat sections for 10' in 3% hydrogen peroxide in PBS.
7. Wash the slides 3X with PBST (0.05% Tween-20) for 5' each at room temperature.
8. Block with avidin/biotin as necessary.
9. Block for 30' at ROOM TEMPERATURE in humidified chamber with blocking buffer (10% normal goat serum; 2X casein/PBS).
10. Blot off the blocking buffer and add rabbit anti-GFP at 1/1000 (Santa Cruz Biotech sc-8334) diluted in dilution buffer (4% goat serum, 1x casein, PBS). Incubate overnight at 2-8°C then for 60' at 37°C next day.
11. Remove excess antibody and wash 3X with PBST for 5' each with gentle shanking.
12. Incubated with biotinylated goat anti-rabbit IgG (Vector Labs, BA-1000) for 30' at ROOM TEMPERATURE (1/200 in PBS).
13. Blot off the excess reagent and wash 3X with PBST for 5' each with gentle shaking.
14. Incubate with streptavidin-peroxidase for 20 minutes at ROOM TEMPERATURE.
15. Blot off the excess reagent and wash 3X with PBST for 5' each with gentle shaking.
16. Incubate with AEC or NovaRed developer and stop the reaction by rinsing in DI H₂O.
17. Counterstain with hematoxylin and mount with coverslip for analysis.