Rb α Arc / Rb α Cre Immunohistochemistry Protocol
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04.02.10

Notes

1. Individual antibodies work well by themselves
2. Ms antibody of each does not work w/ fluorescence
3. Label the least abundant Ab first. If you label w/ the Cre Ab first, I saw a lot of leak through.
4. AffiniPure Fab Fragment Goat Anti-Rabbit IgG (H+L) 111-007-003
5. Cy2-conjugated AffiniPure Fab Fragment Goat Anti-Rabbit IgG (H+L) 111-227-003

Day 1

1. Wash 3X in 1X PBST
    a. Triton will limit background staining
2. Block in 10% NDS for 30 min
    a. Normal serum should be the same species of which the secondary antibody is raised.
    b. 1 ml NDS + 9 ml PBST

3. Primary Ab O/N at 4 C
    a. Rb anti-Arc (1:1000)
    b. 10 ul In 1 ml PBS

Day 2

4. Wash 3X in 1X PBS

5. Secondary Ab at RT for 2 h
    a. Donkey anti-Rb-Cy3 (1:250)
    b. 10 µl in 2.5 ml of PBS

6. Wash 3X in 1X PBS.

7. Incubate in Normal Rabbit Serum in PBS for 2 h (the purpose of this step is to saturate open binding sites on the first secondary antibody with IgG so that they cannot capture the second primary antibody)
    a. 1 ml NRS and 9 ml 1X PBS = 10 ml

8. Wash 3X in 1X PBS

9. Incubate with an excess of Unconjugated Fab donkey anti-rabbit IgG (H+L) (711-007-003, Jackson ImmunoResearch) for 2 h at RT (0.12 mg/ml should be sufficient)
    a. Stock = 1.3 mg / ml
    b. 1 ml of stock + 7 ml PBS = 0.1625 mg / ml
    c. the host species of the Fab antibody should be the same as the host species of the conjugated secondary antibody; this step converts the rabbit IgG so that the second secondary antibody will not bind to it

10. Wash 3X in 1X PBS

11. Second Primary Ab O/N at 4 C
    a. Rb anti-Cre (1:1000)
    b. 1 ul in 1 ml PBS

Day 3

12. Wash 3X in 1X PBS

13. Second Secondary Ab
    a. Donkey anti-Rb-Cy2 (1:250)
    b. 10 ul in 2.5 ml of PBS

14. Wash 3X in 1X PBS