

## Immunohistochemistry for PPAR $\alpha$

1. Deparaffinize sections in:
  - a. xylenes for 5 minutes
  - b. xylenes for 2 minutes
  - c. 100% ethanol for 3 minutes
  - d. 70% ethanol for 3 minutes
  - e. 50% ethanol for 3 minutes
  - f. dd H<sub>2</sub>O for 5 minutes
2. Rinse the slides in 1x PBS twice for 5 minutes
3. Block endogenous peroxidase activity for 30 minutes in: (make fresh)
  - 240ml methanol
  - 5 ml dd H<sub>2</sub>O
  - 6 ml 30% hydrogen peroxide
4. Rinse slides in 1x PBS three times for 5 minutes. Wipe excess liquid off and use a PAP pen to draw around the section on the slide.
5. Block non-specific antibody in blocking buffer for 45 minutes
  - Blocking buffer:
  - 1g BSA, globulin free (SIGMA)
  - 300ul Triton X-100
  - 5ml Horse Serum (Vector Laboratories, #S-2000)
  - QS with PBS to 100ml
  - Filtered using 50ml steriflip filter system (Fisher Scientific)
6. Incubate the slides with the primary antibody in blocking buffer for 1 hr at RT (do not allow to dry out).
  - PPAR C-20 antibody from Santa Cruz diluted 1:200
7. Rinsed 3x with PBS for 5 minutes
8. Incubate the slides with the secondary antibody in blocking buffer at RT for 45 minutes to 1 hour. 1:200 biotinylated anti-goat
9. Rinsed 3x in PBS for 5 minutes
10. Incubate the slides in Streptavidin (ZYMED, #50-420) for 10 minutes at RT.
11. Rinse the slides in 1x PBS 3x for 5 minutes
12. Incubate the slides with AEC (ZYMED, #00-111) at RT for approx 2 minutes.  
Note: Watch the color change and stop incubation based on the intensity of color. Consistency of this incubation step is essential for comparison purposes.
13. Rinse the slides in dd H<sub>2</sub>O for 3minutes

14. If you want to counter-stain, use methyl green or another non-nuclear stain.

15. Mount slides with a cover slip using 3 drops of glycerol gelatin (SIGMA GG-1). Melt glycerol in microwave for 30 seconds before use. When mounting slide, use caution not to seal bubbles under the cover slip.