

PCNA IMMUNOHISTOCHEMISTRY

Optional: heat slides to 68 C for 2 hours

I. Deparaffin (in hood):

- a. HistoClear I 5 min or dip 50 X
 - b. HistoClear II 5 min.
 - c. 100% EtOH I 5 min.
 - d. 100% EtOH II 5min.
 - e. 95% EtOH 3 min.
 - f. 70 % EtOH 3 min.
 - g. 35% EtOH 3min.
 - h. PBS 5 min.
- *slides can be stored @ 4C in PBS o/n at this point
- i. Quench in 3% H₂O₂ in MeOH (stored @ 4 C) for 10 min.
 - j. Wash in PBS 3 X 5 min.

II. Blocking

If PCNA is anti mouse use horse serum to block

- a. set up hydration chamber (Rubbermaid with lid, sponge, H₂O)
- b. drain off excess PBS and dab slides dry
- c. use Pap pen to circle samples
- d. using a pipette tip, apply 5% horse serum in PBS to each sample (150 ul/sample)
- e. incubate for 30 min. @ rt in chamber

III. Primary antibody incubation

- a. drain off blocking solution
- b. using a pipette tip, apply 5% horse serum in PBS, 1:100 PCNA ab-1 (Neomarkers) to *one sample per slide, apply 5% horse serum to the other sample*
- c. incubate 1 hour @ rt or o/n @ 4 C in chamber

IV. Secondary antibody incubation

- a. Wash in PBS 2 X 5 min.
- b. Apply 2% horse serum in PBS, 1:333 secondary antibody (antimouse) to each sample (from MOM kit)
- c. incubate @ rt for 30 min. to 1 hour in chamber
- d. go to step Va-b while incubating

V. ABC Elite vectrastain reagent

- a. Add 2 drops of bottle A and 2 drops of B from Elite Vectrastain ABC kit to 5 ml TS-Tincubate reagents 30 min @ rt (during secondary antibody incubation)

- b. wash slides 2 X in TS-T 5 min
- c. add Elite AB prepared reagent to samples via dropper
- d. incubate 30 min. @ rt in chamber
- e. wash 2 X in TS-T 5 min

VI. Substrate Incubation

- a. Mix solutions from Vector Substrate kit as instructed (in H₂O) just before use *If using DAB: filter solution through Drummond filter using syringe
- b. Apply filtered substrate to samples and incubate no more than 5 min.
- c. Wash 5 min in H₂O; color depends on substrate; DAB=brown, Novared=red (Novared works well)

VII. Counterstain

- a. Counter stain slides with Hematoxylin (Vector) when using a red or brown substrate 2-5 min.
- b. Rinse 1X in H₂O
- c. Rinse in Li₂CO₃ (counter staining will change from brown to blue)
- d. Rinse in H₂O

VIII. Dehydration (in hood)

- a. 35% EtOH 3 min.
 - b. 70 % EtOH 3 min.
 - c. 95% EtOH 3 min.
 - d. 100 % EtOH 5 min.
 - e. 100 % EtOH 5 min.
 - f. histoclear II 5 min.
 - g. histoclear I 5 min.
- If color is faint: skip a and b, 95%, 100%, 100% for 20 sec.

IX. Mount slides

- a. apply a drop of cytoseal to sample while slide is still wet
- b. coverslip immediately
- c. dry at least 30 min. before using microscope

TS-T Buffer

50 ml	1 M tris pH 7.6
30 ml	5 M NaCl
10 ml	10% tween 20 or 1 ml tween 20
910 ml	ddH ₂ O

*if primary antibody is made from rabbit, and biotinylated anti rabbit IgG is made from goat, use 5% goat serum in PBS to block

*if primary antibody is mouse, use horse serum because biotinylated anti mouse IgG is from horse