

**IMMUNOHISTOCHEMISTRY
(for T121)**

I. Deparaffin (in hood):

- a. HistoClear I 5 min
- 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

II. Treatment (in hood)

- a. Boil slides in (700 ml) 1 X citrate buffer for 15 min.; citrate buffer (stored @ 4 C) is 10 X
- b. Remove and incubate @ room temp. 30 min. to cool
- c. Wash in PBS 5 min
- d. Quench in 3% H₂O₂ in MeOH (stored @ 4 C) for 10 min.
- e. Wash in PBS 3 X 5 min.

III. Blocking

SV40 is anti mouse therefore, 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

IV. Primary antibody incubation

- a. drain off blocking solution
- b. using a pipette tip, apply 5% horse serum in PBS, 1:100 SV40 mouse monoclonal primary antibody (Oncogene) to *one sample per slide, apply 5% horse serum to the other sample*
- c. incubate 2 hours @ rt or o/n @ 4 C in chamber

V. 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
- c. incubate @ rt for 30 min. to 1 hour in chamber

- d. go to step VI-a/b while incubating

VI. ABC reagent

- a. Add 2 drops of bottle A and 2 drops of B from Elite Vectrastain ABC kit to 5 ml TS-T
- b. incubate reagents 30 min @ rt (during secondary antibody incubation)
- c. wash slides 2 X in TS-T 5 min
- d. add elite AP prepared reagent to samples via dropper
- e. incubate 30 min. @ rt in chamber
- f. wash 2 X in TS-T 5 min

VII. Substrate Incubation

- a. Combine chemicals from DAB kit as instructed (in H₂O)
- b. Filter solution through Drummond filter using syringe
- c. Apply filtered DAB solution to samples and incubate no more than 5 min.
- d. Wash 5 min in H₂O – color should be brown

VIII. Counterstain

- a. Counter stain in methyl green for 12 min
- b. Rinse 1X in H₂O
- c. Wash 5 min in H₂O

IX. 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.

X. Mount slides

- a. apply drop of cyto seal to sample
- 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
1 ml	tween 20
910 ml	ddH ₂ O