PROTOCOL OF PARAFFIN SECTIONS FOR IMMUNOSTAINING
by JYP 8/13/02

Deparaffinization

Use glass sink
1. Dunk the section in Xylene, 5 minutes X 3 times.
2. Dunk in 100% ETOH, 5 minutes X 2 times.
3. Dunk in 90% ETOH, 5 minutes X 2 times.
4. Dunk in ddH_2O X 1.
5. Boil the section in citrate buffer @ 95°C X 10 minutes.
6. Cool down @ RT X 30 minutes.

Fixation

1. Dunk the section in cold acetone X 5 minutes.
2. Dunk the section in acetone/chloroform (1:1) X 5 minutes.
3. Dunk the section in acetone X 5 minutes.
4. Wash with PBS, 2 minutes X 2.
5. Circle the section with Pap-pen.
6. Place slide in a humidity chamber (by placing wet paper under the slide) with section covered by PBS.

Primary and Secondary antibody incubation

1. Cover the section with the primary antibody (diluted in goat serum: 1:250) X 1-3hr. @ RT or O.N.
2. Wash with PBS, 2 minutes X 3.
3. Incubate the section with goat serum X 10 minutes.
4. Cover the section with secondary antibody (diluted in goat serum: 1:1000) X 1-3hr. @ RT.
5. Wash with PBS, 2 minutes X 3.

DAB staining

1. Cover the section with SA-HRP (diluted in goat serum: 1:500) X 30 minutes.
2. Wash X 2 with PBS.
3. Cover the section with DAB mixture*; monitor the color development (dark brown).
4. Wash with PBS X 3minutes X 2.
5. Counterstain with Light Green working solution, monitor the color change (best result should have both light green and dark brown).
6. Dehydrate by alcohol: Cover the section with 95% ETOH X 5 minutes, remove; Cover the section with 100% ETOH X 5 minutes, remove; then Xylene X 5 minutes X 2.
7. Mount the slide with permount, cover the section with cover slip.

*1X DAB mixture is made by: 10 X DAB/H_2O_2 solution.