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Whole-mount Lectin Staining using Vector Elite ABC Kit

(modified per 9 Aug 94 / AH protocol)

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Experiment Day

After cleaning excess connective tissue, pin specimens onto Sylgard dishes and elevate tissue slightly making sure fluid can pass underneath.

· PBS·0.3% Triton-X preparation: Make up PBS and mix 500 ml PBS with 1.5 ml Triton-X 100 in a beaker. Stir and wait for Triton to go into solution.

Wash tissue with **PBS·0.3% Triton-X 100** *overnight*, and place on rotating shaker.

Next Day

1) **Prepare ABC** (Avidin Biotin Complex) solution. Use reagents A and B from the Vector Elite kit (PK 6101).

· ABC solution preparation: the proportion of both the reagents to the PBS-Triton buffer is 100µl reagent : 25 ml buffer

For example: For two Petri dishes, each which holds 30 ml, measure out 60 ml **PBS·0.3% Triton-X 100** in a graduated cylinder. To it add 240 µl **reagent A** and swirl. Add 240 µl **reagent B** and swirl. (Remove dropper and add reagents to cylinder by micropipette). Let A mix with B in the buffer for 30 minutes before use.

2) **ABC Incubation**: Empty wash, rinse once with additional PBS-Triton, then pipette 30 ml ABC solution into each dish. Let incubate for *22-24 hours*.

Second Day

1) **Tris-Triton washes**: When ABC reaction is nearing completion, have the following solution prepared:

· **Tris Buffer (0.05M) · 1% Triton-X 100**: Using dH_2O, fill beaker close to the total volume of 500 ml. Add 0.97 g Tris base and 6.11 g Tris acid. Bring total volume up to 500 ml. Add stir bar and

stir. Check to make sure pH is approx. 7.2-7.6. While stirring, add 5 ml of Triton-X 100 and allow to mix thoroughly (30 min).

- Wash tissue 4-5 times over 2 hours with the Tris Buffer (0.05%) · 1% Triton.

2) In preparation for DAB gather:

- Fresh diapers and place them in the hood and on the shaker.
- 100 ml disposable beaker.
- Timer
- Waste beaker
- 1% H_2O_2: Add 1 ml 30% H_2O_2 (in fridge) by pipette to 29 ml H_2O in a graduated cylinder.

Afterwards, set pipette dial to amount needed to give 1% of total DAB solution (e.g. for 60 ml DAB solution, set dial for 600µl.)

3) **0.05% DAB in Tris buffer**:

Note: carcinogen. Take care to eliminate contamination of lab by keeping solution in hood and neutralizing with bleach anything that comes in contact with the DAB solution.

- The pellet form of DAB (in fridge) is equivalent to 1 pellet = 10 mg. You want 10 mg / 20 ml Tris buffer.

For example: For two dishes, pour 60 ml Tris Buffer (0.05%)·1% Triton into the beaker and add to it 3 pellets of DAB. Add stir bar and stir until pellets go into solution.

4) **DAB pre-incubation**: After DAB pellets have gone into solution, dump last Triton / Tris wash and add by pipette 15 ml DAB solution to each Petri dish. This will save half of the DAB solution for the next step. Note time; let incubate for 5 mins.

5) **Reaction Substrate**: while incubating, add appropriate amount of H_2O_2 to the remaining DAB solution in the beaker (1% of total DAB volume: eg. for 60 ml DAB, add 600µl 1% H_2O_2).

6) **Incubation**: Split the DAB-H_2O_2 solution (15 ml / dish) and pipette into each dish containing the original DAB. Let sit for 10 min while you marvel at the miracle of browning.

7) **Wash**: Carefully pipette off DAB solution from petri dishes and put into large beaker with bleach. Follow with 2 quick dH_2O tissue washes and several longer (5-10 min) ones, pipetting the waste into the bleach-containing beaker .

8) **EtOH workup**: Dehydrate the tissues in the following series: 50%, 70%, 95%, 100%; let sit for 30 min- 1 hour / stage. (If tissues are of the smashing variety, remove after 70% EtOH).

9) Flatten tissue in a sandwich between two plastic slides which are, in turn, pressed together by two glass slides. Pinch together with clothespins and place in 100% EtOH tubs. Make sure bubbles are not trapped between the slide and tissue.