

## **Anti-*Wolbachia* surface protein monoclonal antibody Immunohistochemistry**

Adult female *Brugia malayi* were fixed in 10% neutral buffered formalin. The worms were paraffin embedded and 5 micron sections were mounted onto glass slides and kept at room temperature until use.

Bring all reagents to room temp before proceeding. All incubations except the primary antibody incubation are carried out at room temp.

1. Bake slides 60° C for 40 min.
2. Deparaffinize and rehydrate only slides that will be processed for this particular experiment
  - a. Xylene substitute bath 5 minutes
  - b. Repeat a
  - c. Tap off excess liquid
  - d. 100% ethanol bath 3 minutes
  - e. repeat d
  - f. tap off excess liquid
  - g. 95% ethanol bath 3 minutes
  - h. repeat g
  - i. tap off excess liquid
  - j. 0.1M PBS bath minimum 30 seconds
3. Outline area of slide the contains sections with wax pencil
4. Block 10 min with 0.05% PBST (0.1M PBS + 0.05 % Tween-20) plus 20% fetal bovine serum
5. Incubate 30 minutes with Anti-WSP mAb at 37° C (optimal dilution from P. Lammie 1:250 in 0.05% PBST plus 20% fetal bovine serum). Note: Incubate for 60 min if done at room temperature.
6. Incubate negative control with same concentration in same diluent rabbit anti-deer Ab
7. Rinse gently with wash solution: 0.05 mol/L Tris-HCL, 0.15 mol/L NaCl, 0.05% Tween 20
8. Add biotinylated swine anti-mouse 2° Ab (yellow) this is the link- incubate 10 min
9. Gently rinse with wash solution
10. Add streptavidin-alkaline phosphatase (red)- incubate 10 min
11. Rinse gently with dH<sub>2</sub>O
12. Add permanent red chromogenic substrate (30 min)
  - a. Reagent preparation (prepare immediately before use)
    - i. Dispense 3 mL of Permanent Red Buffered Substrate into a test tube
    - ii. Add one Permanent Red tablet into the tube
    - iii. Mix well
  - b. Staining procedure
    - i. Tap off excess liquid
    - ii. Cover specimens with Permanent Red solution
    - iii. Incubate 30 minutes
    - iv. Incubate the negative control slide for 30 min
13. Rinse gently with dH<sub>2</sub>O

14. Counterstain with Mayer's hematoxylin
  - a. Stain 2 min
  - b. Rinse with dH<sub>2</sub>O until stain turns blue (~1 min)
15. Immerse slides in 37mM ammonia H<sub>2</sub>O (ammonium hydroxide) (0.037 mol/L)
16. For optimal permanent mounting:
  - a. 1 min, 2 changes each
    - i. 95% ethanol
    - ii. 100% ethanol
    - iii. xylene substitute
17. Mount with Faramount
  - a. NOTE: Faramount remains liquid until dried, thus permitting the user to coverslip numerous slides in succession
  - b. NOTE: store capped bottle of Faramount inverted to prevent bubbles from gathering in the dispensing tip
  - c. Procedure:
    - i. Place coverslips on blotting paper or paper towel on benchtop
    - ii. Apply a continuous line of mountant along one edge of the coverslip
    - iii. Touch the long edge of the slide to the line of mounting medium on the edge of the coverslip
    - iv. Slowly lower the slide toward the coverslip until the two adhere
    - v. Gently press out any bubbles
    - vi. Turn the slide over and lay it flat so that the coverslip is on top
    - vii. Do not wipe the excess mountant as this helps seal the edges
    - viii. Allow the slides to remain flat overnight before stacking or storing

Reagents:

**0.1 M PBS**

50 ml 10X PBS + 450 ml ddH<sub>2</sub>O

**0.05% PBST (0.1 M PBS + 0.05% Tween 20) + 20% fetal bovine serum (FBS)**

6 ml FBS + 15ul Tween 20 + 24 ml 1X PBS

**1:250 anti-WSP mAb in 0.05% PBST + 20% FBS**

1 ml (0.05% PBST + 20% FBS) + 4 ul Ab

**1:250 rabbit anti-deer Ab in 0.05% PBST + 20% FBS (negative control)**

1 ml (0.05% PBST + 20% FBS) + 4 ul Ab

**37 mM ammonium hydroxide in water**

1.25 ml ammonium hydroxide (28-30%) + 500 ml ddH<sub>2</sub>O

**0.05 mol/L Tris-HCL, 0.15 mol/L NaCl, 0.05% Tween 20 (wash solution)**

1.75 g NaCl + 1.58 g Tris-HCl + 100ul Tween 20 then add ddH<sub>2</sub>O to 200 ml

**Reagent ordering information**

Biotinylated swine anti-mouse secondary antibody, streptavidin-alkaline phosphatase and permanent red chromogenic substrate (DAKO Corporation, Carpinteria, CA cat# K0674 and K0695)

Faramount aqueous mounting medium (DAKO Corporation cat#S3025)

Mayer's hematoxylin (Thermo Scientific, Rockford, IL cat#TA-125-MH)