Immunohistochemistry of adult female *Brugia malayi* sections with mAb WSP

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. Mark the frosted part of your slide with initials in wax pencil
4. Outline area of slide the contains sections with wax pencil
5. Block 10 min with 0.05% PBST (0.1M PBS + 0.05 % Tween-20) plus 20% fetal bovine serum.
6. Incubate 30 minutes with Anti-WSP mAb at 37º 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.
7. Incubate negative control primary antibody (your choice) at the same concentration for the same time period.
8. Rinse gently with wash solution: 0.05 mol/L Tris-HCL, 0.15 mol/L NaCl, 0.05% Tween 20.
9. Add biotinylated swine anti-mouse 2° Ab (yellow) this is the link- incubate 10 min.
10. Gently rinse with wash solution.
11. Add streptavidin-alkaline phosphatase (red)- incubate 10 min.
12. Rinse gently with dH_20.
13. 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

14. Rinse gently with dH_20
15. Counterstain with Mayer's hematoxylin
    a. Stain 2 min
    b. Rinse with dH_20 until stain turns blue (~1 min)
16. Immerse slides in 37mM ammonia H_20 (ammonium hydroxide) (0.037 mol/L)
17. For optimal permanent mounting:
    a. 1 min, 2 changes each
        i. 95% ethanol
        ii. 100% ethanol
        iii. xylene substitute
18. 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 because it helps seal the edges
        viii. Allow the slides to remain flat overnight before stacking or storing

Reagents:

<u>0.1 M PBS</u>
50 ml 10X PBS + 450 ml ddH_2O

<u>0.05% PBST (0.1 M PBS + 0.05% Tween 20) + 20% fetal bovine serum (FBS)</u>
6 ml FBS + 15ul Tween 20 + 24 ml 1X PBS

<u>1:250 anti-WSP mAb in 0.05% PBST + 20% FBS</u>
1 ml (0.05% PBST + 20% FBS) + 4 ul Ab

<u>1:250 negative control antibody in 0.05% PBST + 20% FBS (negative control)</u>
1 ml (0.05% PBST + 20% FBS) + 4 ul Ab

<u>37 mM ammonium hydroxide in water</u>
1.25 ml ammonium hydroxide (28-30%) + 500 ml ddH_2O

<u>0.05 mol/L Tris-HCL, 0.15 mol/L NaCl, 0.05% Tween 20 (wash solution)</u>
1.75 g NaCl + 1.58 g Tris-HCl + 100ul Tween 20 then add ddH_2O 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)

Wuchereria bancrofti Monoclonal Anti-*Wolbachia* Surface Protein (WSP) (BEI catalog # NR-31029)

This exercise can be completed in 2.5- 3 hours.

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