IMMUNOHISTOCHEMISTRY: FOXJ1 Mouse MAB on Paraffin Sections of Mouse Lung Using Vector's M.O.M. Blocking Kit.

A. Deparaffinize Tissue & Rehydrate

1. 3 changes of xylene for 10 minutes each.
2. 2 changes of 100% EtOH for 5 minutes each.
3. 2 changes of 95% EtOH for 5 minutes each.
4. 1 change of 70% EtOH for 5 minutes each.
5. Clear in PBS pH 7.2 for 5 minutes.

B. Block Endogenous Peroxidase Activity

1. Place slides in a glass staining dish containing 180 ml of methyl alcohol and 3 ml of 30% hydrogen peroxide.
2. Incubate at room temperature for 15 minutes.
3. Rinse in PBS, pH 7.2, for 5 minutes.

C. Antigen Retrieval (using a 1000 watt Microwave Oven)

1. Fill plastic coplin jars with 50 ml of 0.01M citrate buffer, pH 6.0.
2. Add tap water to plastic reservoir dish to a level of 1 1/2 to 2" to create a sink.
3. Place coplin jars in reservoir.
4. Place 3-4 slides in each coplin jar. (Do not put more than 4 slides per jar!)
5. Microwave on HI for 4.5 minutes (boil).
6. Check fluid level in coplin jars and add DH_2O if level has evaporated significantly.
7. Microwave at 50% power (simmer) 2 times for 7 min. each, for a total of 14 minutes. Check the level of citrate buffer solution after each 7-minute cycle. Add DH_2O if necessary.
8. Remove the reservoir from the microwave.
9. Remove the coplin jars from the reservoir.
10. Cool on the countertop for 15 minutes.
11. Rinse in distilled water X2 quick changes.
12. Rinse in PBS for 5 min.

D. Immunolabeling

Using the Vector M.O.M. kit (Vector PK-2200), follow the instructions as outlined below. **Note:** This is a modification of the instructions in the kit and is performed as an overlay technique.

- circle sections with pap pen
- add PBS to sections
- place horizontally in humidity tray,
- block 60-90 min with M.O.M. block (10 drops in 5 ml PBS/0.15%Triton)
- rinse 2x 5min PBS/0.15%Triton
- presoak in M.O.M. diluent for 5 min
- incubate with primary antibody in M.O.M. diluent for 30 min
- rinse 5x 5 min
- apply biotinylated secondary antibody for 10 min at 1:250 in M.O.M. diluent

  NOTE: We use biotinylated goat anti mouse IgG1 (Southern Biotech cat no 1070-08 lot G7803-wb73) as a substitute for the "pan" anti-IGg in the kit, since the FOXJ1 MAB is an IgG1.
- rinse 3x 5 min
- apply Vector ABC reagent from M.O.M. kit for 5 min
- rinse 3x 5 min
- move slides to rack in PBS for DAB reaction
- presoak in 0.1M acetate buffer (pH6.0)
- incubate 4 min in DAB reaction mixture (0.095g DAB in 200 ml 0.1M acetate buffer with 1.6g NaCl and 2.0g Nickle sulfate)
- 10 dips in 0.1M tris saline
- 4 min in Tris /Cobalt solution
- 10 dips in water
- counterstain with nuclear fast red for 2 min
- dehydrate/clear/coverslip

Note: We have also had some success using our routine general protocol for immunolabeling and a 4% donkey block instead of the M.O.M. kit, as long as we use an isotype specific anti-mouse IgG.

E. Solutions

PBS
2.56 g Na phosphate monobasic
10.6 g Na phosphate dibasic
72.0 g Sodium Chloride
Dissolve in 1 L water; bring to 8 liters with water, check ph 7.2-7.4

0.2 M PB
184 g Na phosphate dibasic
42 g Na phosphate monobasic
Dissolve in1 L water; bring to 8 liters with water, check ph 7.2-7.4

PBS/TRITON
1 L 0.2 M PB
1 L water
18 g Na Cl
3 ml Triton X-100

10x (1.0M) Tris Saline Buffer
242.2 g Tris 7-9 (Sigma)
90 g NaCl
2 L water

Tris Cobalt
2.4 g Tris 7-9
2.0 g cobalt chloride
400 ml water, check pH 7.2

Nuclear Fast Red
0.1 g Nuclear fast red
5.0 g Aluminum sulfate
100 ml distilled water

Dissolve the nuclear fast red and aluminum sulfate in boiling water. Stir at near boiling for at least 2 hours. Cool, filter, and add a few grains of thymol as a preservative. Filter prior to use with Whatman #4 filter paper.

10x (1.0 M acetate buffer)
164 g Na acetate
1 L water

0.01M Citrate Buffer, pH 6.0

1. Stock solutions
   A. 0.1M Sodium Citrate 29.41 gms/1000 ml
   B. 0.1M Citric Acid 21.01 gms/1000 ml

2. Working solution
   82 ml of solution A
   18-19 ml of solution B (use to bring pH to 6.0)

3. Bring to a volume of 1000 ml with DH_2O