Detection of NFAT2 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
DAB Chromogen
Hematoxylin

Staining Kit: M.O.M. Immunodetection Peroxidase Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-2200

Note: This kit contains all reagents necessary to make the blocking solution, secondary antibody and label complex.

Avidin / Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Mouse Monoclonal Anti-NFAT2 Antibody – ChIP Grade
Abcam Inc
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab2796

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 557273

Staining Procedure

Positive Control Tissue: Spleen and thymus (nuclear factor of activated T-cells)
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker
   Add 500 ml of distilled water to the pan inside the decloaker.
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides*_____
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

   M.O.M Peroxidase Kit
   Exp. Date_____ New Kit: yes / no

6. Apply the blocking reagent from the M.O.M. Kit. Incubate for 1 hour at room temperature.
   (Add 2 drops of the Mouse IgG Blocking Reagent to 2.5 ml of 1X PBS.)

   DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit
   Lot #_____ Exp. Date_____ New Kit: yes / no
   Apply avidin block for 15 minutes at room temperature.
   Quick rinse in 1X wash buffer.
   Apply biotin block for 15 minutes at room temperature.

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BLOCK.

   M.O.M. Diluent: Add 600ul of the Protein Concentrate stock solution to 7.5 ml of 1X PBS. Use this as the diluent for the primary, negative, and secondary antibodies.

8. Apply the primary antibody at a 1:1000 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date_____

For negative control slides, dilute mouse IgG1 control serum so that it's IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:1000 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
Lot #_____ Exp. Date _____

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Apply the secondary antibody from the M.O.M. Kit. Incubate for 10 minutes at room temperature. (Add 10ul of the Biotinylated anti-Mouse IgG Reagent to 2.5 ml of the M.O.M. Diluent).

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

12. Apply the label complex from the M.O.M. Kit. Incubate for 5 minutes at room temperature. (Add 2 drops of Reagent A to 2.5 ml of 1X PBS. Mix. Then add 2 drops of Reagent B and mix. Prepare at least 30 minutes prior to use.)

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature. (Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp. Date_____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

Updated 02/26/07