Detection of F4/80 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
0.05 M TrisHCl
DAB Chromagen
Hematoxylin

Antibody Information

Enzyme Retrieval: Trypsin
Sigma-Aldrich
St. Louis, MO, USA
www.sigmaaldrich.com
1-800-325-3010
Catalog# T-4665

Blocking Serum: Normal Rabbit Serum
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog# PK6104

Primary antibody: Rat anti-mouse F4/80 antigen
Caltag Laboratories
Burlingame, CA 94010
www.caltag.com
1-800-874-4007
Catalog# MF48000

Negative Serum Control: Normal Rat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #012-000-001

Secondary antibody: Rabbit anti-rat
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog# BA-4001

Label antibody: Vector EliteVectastain® ABC
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Staining Procedure
Positive Control Tissue: spleen (dendrocytes)
Stain Localization: Cytoplasmic / cell membrane

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

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

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Pre-warm slides in 1X automation buffer at 37°C for 5 minutes.
Incubate the slides in a 0.02% Trypsin, 0.05M Tris-HCl solution at 37°C for 30 minutes.
[DO NOT add the trypsin to the 0.05M Tris-HCl solution until 5 minutes prior to incubation. Trypsin looses 75% of its reactivity within 30 minutes.]
-Stop reaction by rinsing slides in distilled water for 1 min.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.

5. Block with 5% Normal Rabbit Serum and incubate for 20 minutes at room temperature.
Lot#_____ Reconstituted Date_____

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

6. Apply primary antibody (F4/80) at a 1:25 dilution and incubate for one hour at room temperature.
Lot#_____ Exp. Date_____

For negative control slides, normalize the protein concentration of normal rat serum to the protein concentration of the primary antibody (F4/80) and use this to make the 1:25 dilution. Apply normal rat serum to the slides and incubate for one hour at room temperature.
Lot#_____ Reconstituted Date_____

7. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

8. Apply secondary antibody (Biotinylated rabbit anti-rat) at 1:200 dilution and incubate for 30 minutes at room temperature.
Lot#_____ Reconstituted Date_____

9. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

10 Apply label antibody and incubate for 30 minutes at room temperature.
Lot#_____ Exp. Date _____ New Kit: yes / no

11. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

12. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot#_____ Exp. Date_____ New Kit: yes / no

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

16. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

17. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

18. Coverslip.

Updated 05/03/06