Identification of ED2 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:
1X Automation Buffer
3% Hydrogen peroxide
Antibody Diluent
0.05 M TrisHCL pH 7.8 with 1% calcium chloride
DAB Chromagen
Hematoxylin

Antibody Information

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-001

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

Primary antibody: Mouse anti-rat ED2
Serotec, Inc.
Raleigh, NC 27604
1-800-265-7376
www.serotec-inc.com
Catalog MCA342R

Negative Serum Control: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Secondary antibody: Goat anti-mouse IgG HRP
Pierce Corp
www.piercenet.com
Cat# 31430

Staining Procedure

-Positive Control Tissue: liver (Kupffer Cells) and spleen (dendrocytes)

-Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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. Incubate the slides in 0.1% trypsin and incubate at 37°C for 20 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 at 37°C.)
Rinse slide in distilled water for one min to stop reaction.

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

5. Block with 5% Normal Goat Serum and incubate for 20 minutes.
Lot #_____Reconstituted Date_____

6. Apply Avidin/Biotin block
Kit Lot#_____ Exp Date_____ New Kit: yes / no
Apply avidin block - 15 min at RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min at RT.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (ED2)at 1:100 and incubate for one hour.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (ED2) and use this to make the 1:100 dillutions. Apply normal mouse serum to the slides and incubate for one hour.
Lot #_____Reconstituted Date_____

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

9. Apply secondary antibody (Goat anti-mouse --HRP) at 1:50 dilution and incubate for 30 minutes.
Lot #_____ Reconstituted Date_____

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

11. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot#_____ Exp Date_____

12. Rinse in tap water 3 minutes.

13. Counterstain with Modified Harris Hematoxylin for 30 seconds.

14. Rinse in tap water until water is clear.

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

16. Dehydrate through the following solutions.

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

17. Coverslip.
Update 07/28/04