Identification of VCAM in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Dilutions:

Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog# 008-000-001

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

Primary antibody: Goat anti-VCAM (C-19) Antibody
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog #sc-1504

Negative Serum Control: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 005-000-121

Secondary antibody: Biotinylated Horse anti-goat IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-9500

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

Staining Procedure

Positive Control Tissue: Activated endothelial cells of LPS-treated lung
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. Perform Heat Induced Epitope Retrieval using Microwave Oven.
Place a full rack of slides in container containing 200ml 1X citrate buffer.
MWO for 5 minutes at power level 5
Cool for 1 minute (Add citrate buffer to container, if necessary)
MWO for 5 minutes at power level 5. Temp after microwaving_____
Cool 20 minutes at room temperature
Rinse in distilled water for 3 minutes. Repeat twice.

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

5. Apply 10% Normal Horse Serum for 20 minutes at room temperature.
Lot#_____ Reconstituted Date_____

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

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

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (VCAM) at a 1:50 dilution and incubate for one hour at room temperature.
Lot#_____ Exp Date _____

For negative control slides, normalize the protein concentration of normal goat serum to the protein concentration of the primary antibody and use this to make a 1:50 dilution. Apply to the slides and incubate for one hour at room temperature.
Lot#_____ Reconstituted Date_____

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

9. Apply secondary antibody (Horse anti-goat) at a 1:1000 dilution and incubate for 30 minutes at room temperature.
Lot#_____ Reconstituted Date_____

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

11. Apply Label antibody from Vector Elite Kit and incubate for 30 minutes at room temperature. Prepare 30 minutes prior to use.
Lot #_____ Exp. Date _____

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

13. 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

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

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

18. Dehydrate through the following solutions.

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

19. Coverslip

Updated 04/06/06