

Detection of MAdCAM-1 in Formalin Fixed Paraffin-Embedded Mouse Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information

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-mouse mMAdCAM-1 Antibody
R&D Systems
Minneapolis, MN 55413
www.rndsystems.com
1-800-343-7475
Catalog # AF993

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: Peroxidase-conjugated Streptavidin SS Label

Biogenex
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Spleen
Stain Localization: Cytoplasmic (primarily around the germinal centers)

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. Unmasking technique using the decloaker.
Add 500 ml distilled water to the pan of the decloaker.
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.
Decloak for 5 minutes. Pressure _____
Depressurize for 10 minutes.
Remove pan top and cool for 10 minutes. Temperature before cooling _____
Rinse in distilled water twice for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Block with 10% Normal Horse Serum for 20 minutes at room temperature.
Lot# _____ Reconstituted Date _____

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

6. Apply the Avidin Biotin Blocking Kit

Lot# _____ Exp Date _____ New Kit: yes / no

Apply avidin block - 15 minutes at room temperature.

Quick rinse in 1X Automation Buffer.

Apply biotin block - 15 minutes at room temperature.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (MAdCAM-1) at a 1:500 dilution and incubate for one hour at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal goat serum to match the protein concentration of the primary antibody (MAdCAM-1), and use this to make a 1:500 dilution. Apply to 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 Horse anti-goat secondary antibody at a 1:500 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 Biogenix SS Label and incubate for 30 minutes at room temperature.

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 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation buffer until they turn blue.

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 10/17/06