

# Identification of ICAM (M-19) in Formalin-Fixed, Paraffin Embedded Rodent Tissue

## Reagents:

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

## Antibody Information

Blocking serum: Normal Donkey Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
cat# 017-000-011

## Avidin Biotin Blocking Kit

Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog #SP-2001

## Primary antibody: Goat anti-ICAM (M-19)

Santa Cruz Biotechnology, Inc.  
Santa Cruz, CA 95060  
[www.scbt.com](http://www.scbt.com)  
1-800-457-3801  
Catalog #sc-1511

## Negative Serum Control: Normal Goat Serum

Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #005-000-121

Secondary antibody: Donkey anti-Goat  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog# 705-005-147

Label: Vector Standard Elite Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666

**Staining Procedure:**

- Positive Control Tissue: alveoli wall of LPS treated lung
- Stain Localization: cytoplasmic

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. Unmasking Techniques using the decloaker.  
Add 500 ml D/W 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 min. Temp \_\_\_\_\_  
Rinse in D/W, 2x for 3 min each
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.
5. Apply a 10% Normal Donkey Serum block and incubate 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 at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Goat anti-ICAM) at a 1:200 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 (ICAM) and use this to make the 1:200 dilution. Apply normal goat serum 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 Donkey anti-goat IgG 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 label complex and incubate for 30 minutes at room temperature. (Prepare 30 minutes prior to use)

Exp. Date \_\_\_\_\_ New Kit: yes / no

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% EtOH   | 3 changes | 3 minutes |
| Xylene      | 2 changes | 5 minutes |

19. Coverslip  
updated 04/18/05