

# Detection of CD4 in Formalin Fixed, Paraffin-Embedded Human Tissue

## Reagents:

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

## Antibody Information:

### Kit: Mouse Elite Kit

Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog: PK-6102

Note: The Vector Mouse Elite Kit contains solutions needed to make the block, secondary and label antibodies.

### 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: Mouse Monoclonal CD4 Ab-8 (Clone 4B12)

Neomarkers / Lab Vision  
Fremont, CA 94539  
[www.labvision.com](http://www.labvision.com)

1-800-828-1628

Catalog # MS-1528-S

### Negative control serum: Normal Mouse Serum

Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 015-000-001

## Staining Procedure

Positive Control Tissue: Human tonsil

Stain Localization: Membrane

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 500ml distilled water to the pan of the decloaker.  
Place a full rack of slides in a Tissue Tek™ container containing 250ml of 1X EDTA solution.  
Decloak for 5 minutes. Pressure \_\_\_\_\_  
Depressurize for 10 minutes.  
Remove pan top and cool for 10 minutes. Temperature \_\_\_\_\_  
Rinse in distilled water two times for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Apply block from Vector Mouse Elite Kit and incubate for 20 minutes at room temperature.  
Exp Date \_\_\_\_\_ New Kit: yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

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.  
Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (CD4) at a 1:10 dilution and incubate for one hour at room temperature.

Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (CD4). Use this to make a 1:10 dilution 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 from Vector Mouse Elite Kit and incubate for 30 minutes at room temperature.

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

11. Apply label antibody from Vector Mouse Elite Kit and incubate for 30 minutes at room temperature.

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% alcohol  | 1 times | 3 minutes |
| 100% alcohol | 3 times | 3 minutes |
| Xylene       | 2 times | 5 minutes |

19. Coverslip

*Updated 05/24/06*