

Detection of CD25 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 IgG Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6102

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

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

Primary antibody: Mouse Monoclonal Antibody to CD25
Neomarkers / Lab Vision
Fremont, CA
www.neomarkers.com
1-800-828-1628
Catalog # MS-1088-S0

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

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: Human tonsil

Stain Localization: Cell membrane of activated T-cells

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 Techniques using the microwave oven.
Place a full rack of slides in tissue tek^o container containing 200ml of citrate buffer.
Microwave for 5 minutes at level 5
Cool for 1 minute (Add more distilled water if necessary)
Microwave for 5 minutes at level 5. Temp after Microwaving _____
Cool 20 minutes at room 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 blocking solution from the 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 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 (CD25) 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 match the protein concentration of the primary antibody (CD25) and use this to make a 1:10 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 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 the Biogenex SS Label antibody 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 12/19/06