

Detection of CD8 in Formalin-Fixed, Paraffin-Embedded Human Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information:

Blocking serum: Normal Goat Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 005-000-121

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody: Rabbit Polyclonal to CD8

Abcam

Cambridge, MA 02139

www.abcam.com

1-888-772-2226

Catalog# ab4055-500

Negative control serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #011-000-001

Secondary antibody: Biotinylated goat anti-rabbit IgG

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-1000

Label: Vector Standard Elite Kit

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

Staining Procedure

Positive Control Tissue: Human Tonsil (Cytotoxic T-cell lymphocytes)
Stain Localization: Membrane and some cytoplasmic due to secretion

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 decloaker.
Add 500 ml of distilled water to the pan of the decloaker.
Place full rack of slides in a Tissue Tek™ container containing 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 after cooling _____
Rinse in distilled water two times for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.
5. Block with 10% Normal Goat 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 minutes at room temperature.

Quick rinse in 1X Automation Buffer.

Apply biotin block - 15 minutes at room temperature.

Wipe excess block.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (CD8) at a 1:25 dilution and incubate for one hour at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the primary antibody (CD8), and use this to make a 1:25 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 (Goat anti-rabbit) 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 antibody from Vector Elite Kit to one set of slides and the Biogenex Label to the other. 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 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 08/09/06