

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

## Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

### Blocking Serum: 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

### 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: Rabbit Polyclonal Antibody to CD8

Abcam Inc

Cambridge, MA 02139

[www.abcam.com](http://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](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 011-000-001

### Secondary Antibody: Biotinylated Goat Anti-Rabbit IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # BA-1000

Label Complex: Vectastain Standard Elite ABC Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # PK-6100

**Staining Procedure**

Positive Control Tissue: Tonsil (cytotoxic T-cell lymphocytes)

Stain Localization: Cell membrane and some cytoplasmic due to secretions

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* \_\_\_\_\_

Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* \_\_\_\_\_

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

6. Block with 10% Normal Goat Serum for 20 minutes at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

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

7. Avidin / Biotin Blocking Kit

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

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

ONLY WIPE EXCESS BUFFER.

8. Apply primary antibody at a 1:25 dilution, and incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Date Aliquoted \_\_\_\_\_

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:25 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

8. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

9. Apply the goat anti-rabbit secondary antibody at a 1:500 dilution, and incubate for 30 minutes at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

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

11. Apply the label complex from the Standard Elite Kit, and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)

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

12. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

13. Apply the DAB chromagen, and incubate in the dark for 6 minutes at room temperature. (Add 1 drop of DAB per ml of substrate)

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

14. Rinse the slides in tap water 3 minutes.

15. Counterstain with Harris Hematoxylin for 20 seconds.

16. Rinse the slides in tap water until water is clear.

17. Gently agitate slides in 1X Wash Buffer until they turn blue.

18. Dehydrate through the following solutions:

Solution	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip