

## CD8 [C8/144B]

Concentrated and Prediluted Monoclonal Antibody  
901-3160-053023

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3160 A, C	0.1, 1.0 mL	1:100	Van Gogh Yellow
Predilute	API 3160 AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3160 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 3160 G20	20 mL	Ready-to-use	N/A

### Intended Use:

For In Vitro Diagnostic Use

CD8 [C8/144B] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of CD8 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

### Summary and Explanation:

The CD8 antibody reacts with the 32 kDa CD8 protein. CD8 stains cells with cytotoxic activity, including cortical thymocytes, cytotoxic/suppressor T-cells and a subset of natural killer cells. CD4 and CD8 positive and negative staining are indicative of T-cell neoplasms (1-2). CD4 and CD8 may also be used to differentiate between mycosis fungoides and cutaneous inflammatory processes (3-4). CD8 can be used in panels with CD4, CD56, TIA-1 to aid in identifying subsets of inflammatory skin diseases (5). Recently, CD8 has been used in panels with CD103, FOXP3, and PD-1 for the identification of CD8+ tumor infiltrating lymphocytes and their potential value for immune therapy (6-8).

### Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** C8/144B

**Isotype:** IgG1/kappa

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** CD8

**Cellular Localization:** Cell surface

**Positive Tissue Control:** Tonsil and normal colon

### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

### Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

### Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM3160 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).

**Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

**Protein Block (Optional):** Incubate for 10-20 minutes at RT with Val Background Block.

**Primary Antibody:** Incubate for 30 minutes.

**Secondary:** Incubate for 10 minutes with Val Mouse Secondary.

**Linker:** Incubate for 10 minutes with Val Universal Linker.

**Polymer:** Incubate for 10 minutes with Val Universal Polymer.

**Chromogen:** Incubate for 5 minutes with Val DAB.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

### Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** Incubate for 10 minutes at RT with a secondary probe.

**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

### Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

### Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

### Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI3160 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** CD8

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR2, low pH; 101°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** CD8, 30 min., 25°C



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### Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

### Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA ([www.clsi.org](http://www.clsi.org)). 2011

### Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (9)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (10)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

### Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

### References:

1. Barth TF, *et al.* Primary gastric apoptosis-rich T-cell lymphoma co-expressing CD4, CD8, and cytotoxic molecules. *Virchows Arch.* 2000 Apr; 436(4):357-64.
2. Williamson SL, *et al.* New monoclonal antibodies to the T cell antigens CD4 and CD8. Production and characterization in formalin-fixed paraffin-embedded tissue. *Am J Pathol.* 1998 Jun; 152(6):1421-6.
3. Deguchi M, *et al.* Proliferative activity of CD8(+) T cells as an important clue to analyze T cell-mediated inflammatory dermatoses. *Arch Dermatol Res.* 2001 Sep; 293 (9):442-7.
4. Izban KF, *et al.* Immunohistochemical analysis of mycosis fungoides on paraffin-embedded tissue sections. *Mod Pathol.* 1998 Oct; 11(10):978-82.
5. Harvell JD, Nowfar-Rad M, Sundram U. An immunohistochemical study of CD4, CD8, TIA-1 and CD56 subsets in inflammatory skin disease. *J Cutan Pathol.* 2003 Feb;30(2):108-13.
6. Webb JR, Milne K, Nelson BH. PD-1 and CD103 are widely coexpressed on prognostically favorable intraepithelial CD8 T cells in human ovarian cancer. *Cancer Immunol Res.* 2015 Aug;3(8):926-35.

### References Cont'd:

7. Liu S, *et al.* Prognostic significance of FOXP3+ tumor-infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. *Breast Cancer Res.* 2014 Sep 6;16(5):432.
8. Tumei PC, *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature.* 2014 Nov 27;515(7528):568-71.
9. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
10. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.