

**CD8**

Concentrated and Prediluted Monoclonal Antibody  
Control Number: 901-154-032415

ISO  
9001&13485  
CERTIFIED

<b>Catalog Number:</b>	<b>CM 154 A, C</b>	<b>PM 154 AA</b>	<b>OAI 154 T60</b>
<b>Description:</b>	0.1, 1.0 ml, concentrated	6.0 ml, prediluted	60 tests, prediluted
<b>Dilution:</b>	1:75-1:150	Ready-to-use	Ready-to-use
<b>Diluent:</b>	Van Gogh Yellow	N/A	N/A

**Intended Use:**

CD8 [BC/1A5] 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 a 32 kDa protein equivalent to the CD8 molecule. CD8 stains cells with cytotoxic activity, including cytotoxic/suppressor T-cells and a subset of natural killer cells. CD4 and CD8 positive and negative staining are indicative of T-cell neoplasms. CD4 and CD8 are also used to differentiate between mycosis fungoides and cutaneous inflammatory processes. CD8 is also used in panels with CD3, CD4, CD57 and TIA-1.

**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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

**Source:** Mouse monoclonal

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

**Clone:** BC/1A5

**Isotype:** IgG1

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** CD8

**Cellular Localization:** Cell membrane

**Positive Control:** Tonsil or T-cell lymphoma

**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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

**Protocol Recommendations (manual use):**

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment Solution (recommended):** Diva

**Pretreatment Protocol:**

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water; alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's 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 minutes at RT with a tertiary polymer.

**Protocol Recommendations (manual use) Cont'd:**

**Chromogen:**

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

**Counterstain:**

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

**Protocol Recommendations (ONCORE Automated Slide Staining System):**

OAI154 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

**Protocol Name:** CD8

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

**Dewaxing (DS Option):** DS2

**Antigen Retrieval (AR Option):** AR1, high pH; 103°C

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

**Technical Note:**

This antibody has been optimized for use with Biocare's MACH 4 Universal HRP-Polymer Detection and ONCORE HRP Detection. Other Biocare polymer detection kits may be used; however, users must validate incubation times and protocols for their specific application. Use TBS for washing steps.

**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 protocol are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

**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). 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) (3)

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**Precautions Cont'd:**

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. (4)
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/>.

**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. 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."
4. 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.

**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.