

## CD99

Concentrated and Prediluted Rabbit Monoclonal Antibody  
901-392-050719

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

Catalog Number:	CME 392 A	PME 392 AA	OAI 392 T60	VLTR 392 G20
Description:	0.1 mL, conc.	6.0 mL, RTU	60 tests, RTU	20 mL, RTU
Dilution:	1:50	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Da Vinci Green	N/A	N/A	N/A

### Intended Use:

For In Vitro Diagnostic Use

CD99 [EP8] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of CD99 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:

CD99 antigen, a 32 kD T-Cell surface glycoprotein, is also known as MIC2, E2, 12E7, HuLy-m6 or FMC29. Studies have shown this antigen is expressed on the cell membrane of some lymphocytes, cortical thymocytes, and granulosa cells of the ovary (2-4). Studies have also shown CD99 is expressed by most pancreatic islet cells, Sertoli cells of the testis and some endothelial cells. Mature granulocytes express limited or no CD99 (3). Engagement of distinct epitopes on CD99 rapidly induces T-cell death by a novel caspase-independent pathway. CD99 is a highly restricted cell surface antigen of Ewing's sarcoma and primitive peripheral neuroectodermal tumors; therefore, CD99 may aid in identifying Ewing's sarcoma and peripheral neuroectodermal tumors and aid in the differential diagnosis of small blue cell tumors (2-3).

### 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-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Rabbit monoclonal

**Species Reactivity:** Human

**Clone:** EP8 (previously known as EPR3097Y)

**Isotype:** IgG

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

**Epitope/Antigen:** Synthetic peptide to residues on the C-terminus

**Cellular Localization:** Membrane and cytoplasmic

**Positive Tissue Control:** Pancreas

### 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):

VLTR392 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.

### Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:

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

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

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

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

**Secondary:** N/A

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

**Polymer:** Incubate for 20 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 Reveal Decloaker. Refer to the Reveal Decloaker product 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:** N/A

**Polymer:** Incubate for 20-30 minutes at RT with a secondary-conjugated 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™ Automated Slide Staining System):

OAI392 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** CD99 Rb

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

**Dewaxing (DS Option):** DS2

**Antigen Retrieval (AR Option):** AR2, low pH; 95°C

**Reagent Name, Time, Temp.:** CD99 Rb, 30 min., 25°C

### 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.

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### 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) (5)
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 into contact with sensitive areas, wash with copious amounts of water. (6)
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. Sandrin MS, *et al.* Expression cloning of cDNA clones encoding the human cell surface proteins HuLy-m6 and FMC29. Immunogenetics. 1992; 35(4):283-5.
2. Chan JK, *et al.* The MIC2 antibody 013. Practical application for the study of thymic epithelial tumors. Am J Surg Pathol. 1995 Oct; 19(10):1115-23.
3. Robertson PB, *et al.* 013(CD99) positivity in hematologic proliferations correlates with TdT positivity. Mod Pathol. 1997 Apr;10(4):277-82.
4. Soslow RA, *et al.* MIC2, TdT, bcl-2, and CD34 expression in paraffin-embedded high-grade lymphoma/acute lymphoblastic leukemia distinguishes between distinct clinicopathologic entities. Hum Pathol. 1997 Oct;28(10):1158-65.
5. 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."
6. 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.

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