CD8

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-311-052623

BIOCARE MEDICAL

Catalog Number:	**CRM 311 A, C**	**PRM 311 AA**	**VLTR 311 G20**
Description:	0.1, 1.0 mL, conc.	6.0 mL, RTU	20 mL, RTU
Dilution:	1:50	Ready-to-use	Ready-to-use
Diluent:	Van Gogh Yellow	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
CD8 [SP16] is a rabbit 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:
CD8 is a cell surface glycoprotein member of the immunoglobulin superfamily. CD8 consists of two chains, alpha and beta, which are expressed as a disulphide-linked alpha/beta heterodimer or as an alpha/alpha homodimer on a subset of T-cells, thymocytes and NK cells. The majority of CD8+ T cells express CD8 as alpha/beta heterodimer. CD8 functions as a co-receptor in concert with TCR for binding the MHC class I/peptide complex. The HIV-2 envelope glycoprotein binds CD8 alpha chain (but not beta chain). MHC class I restricted receptor; binds to nonpolymorphic region of class I molecules and may increase avidity of interactions between cytotoxic T-cell and target cell during antigen-specific activation. Studies have shown that CD8 stains cortical thymocytes (70-80%), T-cells (25-35% of mature peripheral T-cells, mostly cytotoxic T-cells); NK cells (30%, which are also CD3 negative). CD8 has been shown to be an important marker to analyze T-cell mediated inflammatory dermatoses and is useful for analysis of mycosis fungoides.

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; others not tested
Clone: SP16
Isotype: IgG
Protein Concentration: Lot specific Ig concentration is not available.
Epitope/Antigen: CD8
Cellular Localization: Cell surface
Positive Tissue Control: Tonsil

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative

Storage and Stability:
Store at 2ºC to 8ºC. 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 2ºC to 8ºC.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTR311 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 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 Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva or Reveal Decloaker. Refer to the Diva or 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 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. 2. This antibody stains normal colonic mucosa.

Protocol Recommendations (Ventana BenchMark ULTRA):
PRM311 is compatible for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 64 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 16 minutes, 36°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

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Limitiations Cont'd:
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). 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_3) 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) (4)
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. (5)
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. 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.
2. Izban KF, *et al*. Immunohistochemical analysis of mycosis fungoides on paraffin-embedded tissue sections. Mod Pathol. 1998 Oct;11(10):978-82.
3. 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.
4. 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."
5. 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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