BIOCARE MEDICAL

ISO 9001&13485 CERTIFIED

CD4 + CD8

Prediluted Multiplex Antibody Reagent

Control Number: 901-3157DS-120315

Catalog Number: **API 3157 DS AA**
Description: 6.0 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use
CD4 + CD8 is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of CD4 and CD8 proteins 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:

CD4 is expressed in a T-cell subset (helper/inducer) and is found in approximately 80% of thymocytes and in 45% of peripheral blood lymphocytes. CD4 is expressed in the majority of T-cell lymphomas including mycosis fungoides, a common form of cutaneous T-cell lymphoma (1).

CD8 has been shown to be an important marker in the analysis of T-cell mediated inflammatory dermatoses and is also useful for analysis of mycosis fungoides (2-4).

CD8 can be used in panels with CD4, CD56, TIA-1 to aid in identifying subsets of inflammatory skin diseases (4). CD4 and CD8 have also been shown to be valuable in squamous cell cervical cancer and gastric mucosa in HIV infection (5-7). The combination of CD4(+) and CD8(-) is helpful in distinguishing mycosis fungoides and can be used in a panel of CD2(+), CD3(+) and CD7(-/+) (1-3). Multiplex IHC may also give distinct advantages if ratios and/or cell counts on a single slide are desired.

Principle of Procedure:

This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:

CD4 + CD8 is provided as a prediluted antibody cocktail of anti-CD4 and anti-CD8 antibodies in buffer with carrier protein and preservative.

Antibody	anti-CD4	anti-CD8
Clone	4B12	SP16
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1/kappa	IgG
Epitope/ Antigen	CD4	CD8
Cellular Localization	Cell surface	Cell surface
Staining	Brown (DAB)	Red (Warp Red)

Storage and Stability:
Store at 2ºC to 8ºC. Do not use reagent after the expiration date printed on the vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity: Human; others not tested

Positive Tissue Control: Mycosis fungoides and normal tonsil

Protocol Recommendations:

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

Pretreatment: Perform heat retrieval using Biocare's Borg Decloaker. Refer to the Borg Decloaker product data sheet for specific instructions.

Protein Block: Incubate for 10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.

Chromogen (1): Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

Chromogen (2): Incubate for 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

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

Technical Notes:

This antibody has been standardized with Biocare's MACH 2 Double Stain 2. Use TBS buffer 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 protocols 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_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) (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.

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Precautions Cont'd:

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. Boone SL, Guitart J, Gerami P. Follicular mycosis fungoides: a histopathologic, immunohistochemical, and genotypic review. G Ital Dermatol Nenereol. 2008 Dec;143 (6):409-14.
2. Hodak E, *et al.* CD4/CD8 double-negative epidermotropic cutaneous T-cell lymphoma: an immunohistochemical variant of mycosis fungoides. J Am Acad Dermatol. 2006 Aug;55(2):276-84.
3. Tirumalae R, Panjwani PK. Origin Use of CD4, CD8, and CD1a Immunostains in Distinguishing Mycosis Fungoides from its Inflammatory Mimics: A Pilot Study. Indian J Dermatol. 2012 Nov;57(6):424-7.
4. 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.
5. Shi Z, *et al.* Frequency, distribution of CD4+, CD8+ T cells and expression of CD38 in gastric mucosa of HIV infections. Za Zhi. 2009 Aug;23(4):261-4.
6. Shah W, *et al.* A reversed CD4/CD8 ratio of tumor-infiltrating lymphocytes and a high percentage of CD4(+)FOXP3(+) regulatory T cells are significantly associated with clinical outcome in squamous cell carcinoma of the cervix. Cell Mol Immunol. 2011 Jan;8(1):59-66.
7. 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.
8. 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.
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.

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