CD4 (M) + CD8 (RM)

Prediluted Multiplex Cocktail (4-Step)

Control Number: 901-395DS-091317

Catalog Number: **PM 395 DS AA**
Description: 6.0 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use

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.

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 in the analysis of T-cell mediated inflammatory dermatoses and is also useful for analysis of mycosis fungoides. CD4 and CD8 have also been shown to be valuable in squamous cell cervical cancer and gastric mucosa in HIV infection. The combination of CD4(+) and CD8(-) are helpful in distinguishing mycosis fungoides and can be used in a panel of CD2(+), CD3(+) and CD7(-/+). Multiplex IHC may also give distinct advantages if ratios and/or cell counts on a single slide are desired.

Principle of Multiplex Staining:
A Multiplex IHC stain can be accomplished in four major steps. The initial step consists of an antibody cocktail with at least one mouse and one rabbit antibody. This cocktail is applied to the tissue and will bind with two or more target antigens. A multiplex detection cocktail of horseradish peroxidase (HRP) and alkaline phosphatase (AP) conjugated secondary antibodies is applied. The third step consists of the addition of DAB-Substrate that binds to the HRP and produces a brown chromogenic reaction product. The fourth step consists of a Fast Red-Substrate that binds to the AP and produces a red chromogenic reaction product.

Source: Mouse monoclonal and Rabbit monoclonal

Species Reactivity: Human, others not tested

Clone: BC/1F6 +SP16

Isotype: IgG1+ Rabbit IgG

Epitope/Antigen: CD4+CD8

Cellular Localization:
CD4: (Cell surface/membrane): Brown
CD8: (Cell surface): Red

Positive Control: Tonsil or mycois fungoides

Normal Tissue: Tonsil

Abnormal Tissue: T-cell lymphoma and mycosis fungoides

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

Supplied As: Buffer with protein carrier and preservative.

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

Protocol Recommendations

Peroxide Block:
(After the Primary Antibody)

Pretreatment Solution (recommended): Borg

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 20 minutes then wash in distilled water.

Protein Block:
Optional: Incubate for 10-20 minutes at RT with BIOCARE's Background Sniper.

Primary Antibody:
Incubate for 30-60 minutes at RT. Block for 5 minutes with BIOCARE'S PEROXIDAZED 1 after the primary antibody. Wash in TBS buffer.

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 when using BIOCARE's Betazoid DAB.

Chromogen (2):
Incubate for 10-20 minutes at RT with BIOCARE's Vulcan Fast Red. Rinse in deionized water.

Counterstain:
Rinse with deionized water. Incubate for 30-60 seconds with Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing solution for 1 minute.

Technical Note:
This antibody has been standardized with BIOCARE's MACH 2 Double Stain 2. It can also be used on an automated staining system. Use TBS buffer for washing steps.

Performance Characteristics:
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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:
Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about Tissue Controls.

Precautions:
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)

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.

Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

CD4 (M) + CD8 (RM)

Prediluted Multiplex Cocktail (4-Step)

Control Number: 901-395DS-091317

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.

Limitations and Warranty:

There are no warranties, expressed or implied, which extend beyond this description. BIOCARE is not liable for property damage, personal injury, or economic loss caused by this product.

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. Shi Z, et al., Za Zhi. 2009 Aug;23(4):261-4. Frequency, distribution of CD4+, CD8+ T cells and expression of CD38 in gastric mucosa of HIV infections.
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. National Committee for Clinical Laboratory Standards(NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080