Product Code: 
MP-395-DS6 (6ml RTU)

Product Description: 
CD4 (M) + CD8 (RM) 
Prediluted Multiplex Cocktail (4-Step)
Control Number: 901-395DS-021010
ISO 9001:2000 CERTIFIED

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.

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.

Intended Use: For In Vitro Diagnostic Use

Source: Mouse Monoclonal and Rabbit Monoclonal
Species Reactivity: Human; others not tested

Clone: BC/1F6 +SP16

Isotype: IgG1 and Rabbit IgG

Epitope/Antigen: CD4+CD8

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

Positive Control: Tonsil or mycosis 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 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.
**Instructions for Use**

**Pre-treatment (Recommended)**
Retrieve sections with MenaPath Access Super Solutions using the MenaPath Access Retrieval Unit 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 sections for 10-15 minutes at room temperature with the MenaPath Casein Background Blocker.

**Primary Antibody**
Incubate for 30-60 minutes at RT. Rinse in buffer.

**Endogenous Peroxidase Block**
Block all endogenous peroxidase activity after the primary antibody by incubating the sections for 5 minutes with the MenaPath Peroxide Block. Rinse slides in water, and then rinse well in buffer.

**Double Stain Detection:**
Incubate for 30 minutes at RT with MenaPath Multiplex Detection Kit 2. Rinse in buffer.

**Chromogen 1:**
Incubate sections for 5 minutes at room temperature with the MenaPath Liquid Stable DAB. Rinse in buffer.

**Chromogen 2:**
Incubate for 10 minutes at RT with MenaPath Fast Red. Rinse in DI Water.

**Counterstain:**
Rinse with deionized water. Incubate for 30-60 seconds with MenaPath Haematoxylin. Rinse with deionized water. Apply Bluing solution for 1 minute.
Technical Note:
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 titres listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of MenaPath 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.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Menarini Technical Support at 0118 944 4100

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.

Precaution
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 (NaN₃) 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 non-specific 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.
Limitations and Warranty:
There are no warranties, expressed or implied, which extend beyond this description. Menarini Diagnostics is not liable for property damage, personal injury, or economic loss caused by this product.

References: