L26 + CD3

Prediluted Multiplex Cocktail (4-Step)

Control Number: 901-237DS-082717

Catalog Number: **PM 237 DS AA**
Description: 6.0 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use

Summary and Explanation:
L26 (CD20) reacts with a 30-33 kDa polypeptide present in B-cells. L26 reacts with the majority of B-cells present in peripheral blood and lymphoid tissues. In normal lymphoid tissue, L26 marks B-cells in germinal centers, particularly immunoblasts. This antibody has been shown to be a reliable pan B-cell marker. It rarely marks T-cells.

This rabbit monoclonal CD3 reacts with the intracytoplasmic portion of the CD3 antigen expressed by T-cells. It stains human T-cells in both the cortex and medulla of the thymus and in peripheral lymphoid tissues.

L26 and CD3 have been cocktailed to be used with Biocare's MACH 2 Double Stain 1. L26 is stained with Fast Red and CD3 is stained with DAB.

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: L26 + SP7

Isotype: IgG_{2a} + Rabbit IgG

Epitope/Antigen: L26: B-cells , CD3: T-cells

Cellular Localization:
L26: cytoplasmic (red), CD3: cytoplasmic (brown)

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

Positive Control: Tonsil

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.

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

Pretreatment Solution (recommended): Borg or Diva

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 10 minutes then wash in distilled water.

Protein Block:
Optional: Incubate for 10-15 minutes at RT with Biocare's Background Sniper.

Primary Antibody:
Incubate for 30-60 minutes at RT.

Double Stain Detection:
Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 1.

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.

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 1. It can also be used on an automated staining system. 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. 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 (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 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.

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.

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References:

1. Rossi S, et al. Rabbit monoclonal antibodies: a comparative study between a novel category of immunoreagents and the corresponding mouse monoclonal antibodies. Am J Clin Pathol. 2005 Aug;124(2):295-302.
2. Nguyen DT, et al. Differential diagnosis of L26-positive, CD15-negative Hodgkin's disease and large B-cell lymphoma with a high content of reactive T-cells: a morphologic and immunohistochemical study. Hematopathol Mol Hematol. 1996;10 (3):135-50.
3. Chadburn A, Knowles DM. Paraffin-resistant antigens detectable by antibodies L26 and polyclonal CD3 predict the B- or T-cell lineage of 95% of diffuse aggressive non-Hodgkin's lymphomas. Am J Clin Pathol. 1994 Sep;102(3):284-91.
4. Davey FR, et al. Immunophenotyping of non-Hodgkin's lymphomas using a panel of antibodies on paraffin-embedded tissues. Am J Pathol. 1987 Oct;129(1):54-63.
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. 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.

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