BIOCARE MEDICAL

D2-40 + CD31

ISO 9001&13485 CERTIFIED

Prediluted Multiplex Antibody Cocktail

Control Number: 901-3021DS-051315

Catalog Number: **API 3021DS AA**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

D2-40 + CD31 [D2-40 + EP78] is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of O-linked sialoglycoprotein D2-40 and CD31 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:

D2-40 is a selective marker of lymphatic endothelium in normal tissues and vascular lesions. Studies have shown D2-40 effectively marked the lymphatic channel endothelium, but not the adjacent capillary. CD31, also known as PECAM-1, is a 130 kDa integral membrane glycoprotein found on the surface of endothelial cells, platelets and some hematopoietic cells. The CD31 antibody also labels endothelial cells of arteries, arterioles, venules, veins, and non-sinusoidal capillaries in various tissues. CD31 has been shown to be one of the most sensitive and specific endothelial cell markers. In addition, CD31 has been used to evaluate vascular invasion of tumors and assess angiogenesis. The combination of D2-40 and CD31 can serve as a co-marker for both lymphatic density and blood vascular studies.

Source: Mouse monoclonal and Rabbit monoclonal

Species Reactivity: Human; others not tested

Clone: D2-40 (D2-40) + EP78 (CD31)

Isotype: IgG1 (D2-40) + IgG (CD31)

Epitope/Antigen: O-linked sialoglycoprotein (D2-40) + a synthetic peptide derived from human CD31

Cellular Localization:

D2-40 (Cytoplasmic, lymphatic epithelium): Brown with MACH 2 Double Stain 2 or Red with MACH 2 Double Stain 1.

CD31 (Cytoplasmic/membrane): Red with MACH 2 Double Stain 2 or Brown with MACH 2 Double Stain 1.

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Positive Tissue Control: Colon cancer

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 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.

Protocol Recommendations:

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

Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva 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 or MACH 2 Double Stain 1.

Protocol Recommendations Cont'd:

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 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 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) (7)
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. (8)
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.

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References:

1. Kahn HJ, Marks A. A new monoclonal antibody, D2-40, for detection of lymphatic invasion in primary tumors. Lab Invest. 2002 Sep;82(9):1255-7.
2. Engel CJ, *et al.* Tumor angiogenesis predicts recurrence in invasive colorectal cancer when controlled for Dukes staging. Am J Surg Pathol. 1996 Oct;20(10):1260-5.
3. El-Gohary YM, *et al.* Significance of periductal lymphatic and blood vascular densities in intraductal carcinoma of the breast. Breast J. 2009 May-Jun;15(3):261-7.
4. Saad RS, *et al*. Lymphatic vessel density as a prognostic marker in clinical stage I endocervical adenocarcinoma. Int J Gynecol Pathol. 2010 Jul;29(4):386-93.
5. El-Gohary YM, *et al.* Prognostic significance of intratumoral and peritumoral lymphatic density and blood vessel density in invasive breast carcinomas. Am J Clin Pathol. 2008 Apr;129(4):578-86.
6. Renyi-Vamos F, *et al.* Lymphangiogenesis correlates with lymph node metastasis, prognosis, and angiogenic phenotype in human non-small cell lung cancer. Clin Cancer Res. 2005 Oct 15;11(20):7344-53.
7. 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."
8. 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.

Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.

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