

## D2-40 + CD31

### Prediluted Multiplex Antibody Cocktail

Control Number: 902-3021DS-051315

**Catalog Number:** APR 3021DS AA

**Description:** 6.0 ml, prediluted

**Dilution:** Ready-to-use

**Diluent:** N/A

**Intended Use:**

For Research Use Only. Not for use in diagnostic procedures.

**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 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 2°C to 8°C. 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.

**Staining Protocol Recommendations:**

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 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.

**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:**

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

**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<sub>3</sub>) 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>.

**Technical Support:**

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

**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.

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