

D2-40 (Lymphatic Marker)

Concentrated and Prediluted Monoclonal Antibody
901-266-101817

BIOCARE
M E D I C A L

Catalog Number:	CM 266 A, B, C	PM 266 AA	IP 266 G10	OAI 266 T60
Description:	0.1, 0.5, 1.0 ml, concentrated	6.0 ml, prediluted	10 ml, prediluted	60 tests, prediluted
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Da Vinci Green	N/A	N/A	N/A

Intended Use:

For In Vitro Diagnostic Use

D2-40 (Lymphatic Marker) [D2-40] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of O-linked sialoglycoprotein 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:

Studies have shown D2-40 reacts with an O-linked sialoglycoprotein (MW 40K) found on lymphatic endothelium, fetal testis and on the surface of testicular germ-cell tumors. D2-40 is also known as a selective marker of lymphatic endothelium in normal tissues and vascular lesions. In a few studies, clone D2-40 has shown staining reaction in lymphatic channel endothelium, but not in the adjacent capillary. Clone D2-40 has also shown staining in endothelium of lymphangiomas whereas hemangiomas, glomus tumors, angioliopomas, pyogenic granulomas and vascular malformations did not show any staining. D2-40 has also been shown to react with Kaposi's sarcoma and a subset of angiosarcomas. Proliferation and distribution of lymphatic vessels may also be related to prognosis and early metastasis. D2-40 has also been shown to be a very specific marker for malignant mesothelioma (1-2).

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human, mouse and rat

Clone: D2-40

Isotype: IgG1

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

Epitope/Antigen: O-linked sialoglycoprotein

Cellular Localization: Cytoplasm (lymphatic epithelium)

Positive Tissue Control: Tonsil, breast cancer or 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 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.

Protocol Recommendations (intelliPATH and manual use):

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 data sheet for specific instructions.

Protocol Recommendations (intelliPATH and manual use)

Cont'd:

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 60 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe.

Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB - OR - Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

intelliPATH™ Automated Slide Stainer:

IP266 is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the intelliPATH Automated Slide Stainer manual for specific instructions on its use. When using the intelliPATH, peroxide block with intelliPATH Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

Protocol Recommendations (ONCORE Automated Slide Staining System):

OAI266 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

Protocol Name: D2-40

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Option): DS Buffer

Antigen Retrieval (AR Option): AR1, high pH; 103°C

Reagent Name, Time, Temp.: D2-40, 30 min., 25°C

Technical Note:

This antibody has been optimized for use with Biocare's MACH 4 Universal HRP-Polymer Detection, intelliPATH Universal HRP Detection Kit and ONCORE HRP Detection. Use TBS 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. 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

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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 (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) (3)
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. (4)
3. Microbial contamination of reagents may result in an increase in non-specific 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.

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. Chu AY, *et al.* Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. *Mod Pathol.* 2005 Jan;18(1):105-10.
3. 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."
4. 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.