

D2-40 (Lymphatic Marker)

Concentrated and Prediluted Monoclonal Antibody
901-266-120921

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M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 266 A, B, C	0.1, 0.5, 1.0 mL	1:100	Da Vinci Green
Predilute	PM 266 AA	6.0 mL	Ready-to-use	N/A
intelliPATH FLX	IP 266 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 266 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 266 T60	60 tests	Ready-to-use	N/A
Q Series— For Leica BOND-III	ALI 266 G7	7.0 mL	Ready-to-use	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, angiolipomas, 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. After labeling the antigen with a primary antibody, a one-step or two-step detection procedure can be applied. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human, mouse, and rat

Clone: D2-40

Isotype: IgG1

Protein Concentration: 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. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with 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 Warp Red.

Counterstain:

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

intelliPATH FLX Automated Slide Stainer:

IP266 is intended for use with the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI266 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the 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

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI266 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: D2-40

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Buffer Option): DS Buffer

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

Block Option: Buffer

Reagent Name, Time, Temp.: D2-40, 1 hour, 25°C

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Protocol Recommendations (Q Series – For Leica BOND-III):

ALI266 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Protocol Name: IHC Protocol F
Detection: Bond Polymer Refine
HIER: 30 min with ER2
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Post Primary: 8 min
Polymer: 8 min
Mixed DAB Refine: 10 min
Hematoxylin: 5 min

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.

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 (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.

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