BIOCARE MEDICAL

ISO 9001&13485 CERTIFIED

D2-40 + CK8/18

Prediluted Multiplex Antibody Reagent

Control Number: 901-3034DS-052815

Catalog Number: **API 3034DS AA**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

D2-40 + CK8/18 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 cytokeratins 8 and 18 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 reacts with an O-linked sialoglycoprotein (MW 40 kDa) found in lymphatic endothelium, fetal testis and on the surface of testicular germ-cell tumors. D2-40 is a new selective marker of lymphatic endothelium in normal tissues and vascular lesions. According to studies, clone D2-40 has shown a staining reaction in lymphatic channel endothelium, but not in the adjacent capillaries. Cytokeratin 8/18 (CK8/18) are type-II Intermediate Filament proteins that are found in many simple epithelial cells in respiratory, gastrointestinal, thyroid, and male and female reproductive tracts. In immunohistochemistry, CK8/18 has also been shown to stain most carcinomas such as liver, prostate, pancreatic, lung, breast and colon cancers. By labeling lymphatic endothelium with D2-40 (DAB) and carcinomas with CK8/18 (Warp Red), the staining contrast in a single section may simplify the evaluation and assessment of lymphatic microinvasion.

Reagent Provided:

D2-40 + CK8/18 is provided as a prediluted antibody cocktail of anti-D2-40, anti-CK8, and anti-CK18 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-D2-40	anti-CK8	anti-CK18
Clone	D2-40	EP17	EP30
Source	Mouse monoclonal	Rabbit monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG	IgG
Epitope/ Antigen	O-linked sialoglycoprotein D2-40	CK8	CK18
Cellular Localization	Cytoplasmic lymphatic endothelium	Cytoplasmic	Cytoplasmic
Staining	Brown (DAB)	Red (Warp Red)	Red (Warp Red)

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.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity: Human

Positive Tissue Control: Normal breast or breast carcinoma

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 30-60 minutes at RT.

Protocol Recommendations Cont'd:

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

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) (4)
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. (5)
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. Saad RS, *et al.* Lymphatic microvessel density as prognostic marker in colorectal cancer. Mod Pathol. 2006 Oct; 19(10):1317-23.
2. Angus B, *et al.* Cytokeratins in cervical dysplasia and neoplasia: a comparative study of immunohistochemical staining using monoclonal antibodies NCL-5D3, CAM5.2, and PKK1. J Pathol. 1988 May; 155(1):71-5.
3. Angus B, *et al.* NCL-5D3: a new monoclonal antibody recognizing low molecular weight cytokeratins effective for immunohistochemistry using fixed paraffin-embedded tissue. J Pathol. 1987 Dec; 153(4):377-84.
4. 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."
5. 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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