



D2-40 (M) + Ki-67 (RM)

Prediluted Multiplex Cocktail (4-Step)

Control Number: 902-399DS-010213

Catalog Number: APR 399 DS 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 new selective marker of lymphatic endothelium in normal tissues and vascular lesions. In recent studies, clone D2-40 has shown a staining reaction in lymphatic channel endothelium, but not in the adjacent capillary. D2-40 has been widely used for tumor lymphangiogenesis and lymphatic vessel invasion in human cancers. The Ki-67 nuclear antigen is associated with cell proliferation and is used to grade proliferation rates of tumors. This Multiplex IHC consists of D2-40 and Ki-67 antibodies which stain D2-40 and Ki-67 proteins respectively.

Source: Mouse monoclonal and Rabbit monoclonal

Species Reactivity: Human, others not tested

Clone: D2-40 + SP6

Isotype: IgG1 + Rabbit IgG

Epitope/Antigen: D2-40 + Ki-67

Cellular Localization:

D2-40 (Cytoplasm/lymphatic epithelium): Red

Ki-67 (Nuclear): Brown

Positive Control: Colon or tonsil

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.

Staining Protocol Recommendations:

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

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water. Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

Protein Block (Optional): Incubate for 10-15 minutes at RT with Biocare's Background Sniper.

Primary Antibody:

Incubate for 30-60 minutes at RT.

Double Stain Detection:

Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 1.

Chromogen (1): Incubate for 5 minutes at RT when using Biocare's Betazoid DAB.

Chromogen (2): Incubate for 10-20 minutes at RT with Biocare's Vulcan Fast Red. Rinse in deionized water.

Counterstain:

Rinse with deionized water. Incubate for 30-60 seconds with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute.

Technical Note:

This antibody has been standardized with Biocare's MACH 2 Double Stain 1. It 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 (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)
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.
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 MSDS is available upon request and is located at <http://biocare.net/support/msds/>.

Technical Support:

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

References:

1. Kadota K, *et al.* The clinical significance of the tumor cell D2-40 immunoreactivity in non-small cell lung cancer. *Lung Cancer*. 2010 Jan 8. [Epub ahead of print]
2. Cassarino DS, *et al.* Primary dermal melanoma: distinct immunohistochemical findings and clinical outcome compared with nodular and metastatic melanoma. *Arch Dermatol*. 2008 Jan; 144(1):49-56.
3. Fernández MI, *et al.* Prognostic implications of lymphangiogenesis in muscle-invasive transitional cell carcinoma of the bladder. *Eur Urol*. 2008 Mar; 53(3):571-8. Epub 2007 Aug 24.
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-Third Edition CLSI document M29-A3 Wayne, PA 2005

