

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

Prediluted Multiplex Cocktail (4-Step)

Control Number: 901-399DS-091214

**Catalog Number:** PM 399 DS AA

**Description:** 6.0 ml, prediluted

**Dilution:** Ready-to-use

**Diluent:** N/A

**Intended Use:**

For In Vitro Diagnostic Use

**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. The Multiplex IHC stain D2-40 and Ki-67 can be used to detect relative lymph vessel area, lymph vessel perimeters, and simultaneously used to calculate cell proliferation rates in tumors, thus is useful in the identification of aggressive types of cancer and their potential metastasis.

**Principle of Multiplex Staining:**

A Multiplex IHC stain can be accomplished in four major steps. The initial step consists of an antibody cocktail with at least one mouse and one rabbit antibody. This cocktail is applied to the tissue and will bind with two or more target antigens. A multiplex detection cocktail of horseradish peroxidase (HRP) and alkaline phosphatase (AP) conjugated secondary antibodies is applied. The third step consists of the addition of DAB-Substrate that binds to the HRP and produces a brown chromogenic reaction product. The fourth step consists of a Fast Red-Substrate that binds to the AP and produces a red chromogenic reaction product.

**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

**Normal Tissue:** Tonsil, breast, colon, lung

**Abnormal Tissue:** Breast, lung, prostate and colon carcinomas

**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.

**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.

**Performance Characteristics:**

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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

**Quality Control:**

Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about tissue controls.

**Precautions:**

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)

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.

Microbial contamination of reagents may result in an increase in nonspecific staining.

Incubation times or temperatures other than those specified may give erroneous results.

The user must validate any such change. The MSDS is available upon request.

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**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.

**Limitations and Warranty:**

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by 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. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991; 7(9). Order code M29-P.