



Mouse anti-Podoplanin (D2-40, Lymphatic Endothelial Marker)

Cat. No.: AIB-30124 (1 ml Concentrate); AIB-30125 (0.5 ml Concentrate); AIB-30123 (6 ml Ready-to-use)

Instructions for use

Intended use

This antibody is designed for the specific localisation of lymphatic endothelial marker Podoplanin in formalin-fixed, paraffin-embedded tissue sections. The antibody is also suitable for Western Blot.

Anti-Podoplanin antibody is intended for in vitro diagnostic use.

Specifications

Specificity: Podoplanin

Immunogen: Oncofetal antigen M2A

Clone: D2-40 Isotype: Mouse IgG1a

Species reactivity: Human +, others not tested

Summary and Description

Podoplanin is a transmembrane mucoprotein (38 kDa) which is recognized by monoclonal antibody D2-40. It is selectively expressed in lymphatic epithelia, in lymphangiomas, Kaposi sarcomas and in some angiosarcomas with supposed lymphatic differentiation. Additionally, it was shown that Podoplanin is also expressed in epithelioid mesotheliomas, hemangioblastomas and seminomas.

Anti-Podoplanin antibody stains positive in the cytoplasm of lymphatic vessel endothelia but not in blood vessel endothelia. Hence, it is helpful for a reliable differentiation of lymphatic vessels from blood vessels and in identification of retraction artefacts in tissue sections. According to Ordonez 2005 and Chu et al. 2005 Podoplanin/D2-40 is a sensitive and specific marker for malignant mesotheliomas.

Reagent provided

Mouse monoclonal antibody from cell culture supernatant in PBS with carrier protein and preservative for stabilisation in the following formats:

 Concentrate:
 1 ml
 (Cat. No. AIB-30124)

 Concentrate:
 0.5 ml
 (Cat. No. AIB-30125)

 Ready-to-use:
 6 ml
 (Cat. No. AIB-30123)

Dilution of primary antibody

Dilution of Nordic Biosite' concentrated antibody depends on the detection system used. The final working dilution must always be determined by the user. The elaboration of staining protocol should be done by an experienced specialist. For Nordic Biosite' recommendations see chapter 'Staining procedure'.

Explanations of the symbols on the product label:

REI	Catalog Number Bestellnummer Reference du catalogue	LOT	Batch Code Chargenbezeichnung Code du lot	Manufacturer Nordic BioSite AB
	Use By Verwendbar bis Utiliser jusque	IVD	In Vitro Diagnostic Medical Device In vitro Diagnostikum Dispositif médical de diagnostic in vitro	Propellervägen 4A S-183 62 Täby Sweden Tel: +46 (0)8 5444 33 40 Fax: +46 (0)8 756 94 90 info@nordicbiosite.com www.nordicbiosite.com
	Consult Instructions for use Gebrauchsanweisung beachten Consulter les instructions d'utilisation		Temperature Limitation Lagerungstemperatur Limites de température	

Storage and handling

The antibody should be stored at 2-8°C without furt her dilution.

Dilutions of the concentrated antibody should be done with a suitable antibody dilution buffer (e.g. BCB-20005/BCB-20006 from Nordic Biosite). The diluted antibody should be stored at 2-8°C after use. Stability of this working solution depends on various parameters and has to be confirmed by appropriate controls. The antibody provided is suitable for use until the expiry date indicated on the label, if stored at 2-8°C. Do not use product after the expiry date. Positive and negative controls should be run simultaneously with all specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Nordic Biosite' technical support or your local distributor.

Precautions

Use through qualified personnel only.

Wear protective clothing to avoid contact of reagents and specimens with eye, skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with large amounts of water. Microbial contamination of the reagent must be avoided, since otherwise non-specific staining may occur. Sodium azide (NaN3), used for stabilisation, is not considered hazardous material in the concentration used. Reaction of sodium azide with lead or copper in drainage pipes can result in the formation of highly explosive metallic azides. Sodium azide should be discarded in a large volume of running water to avoid formation of deposits. Material safety data sheets (MSDS) are available upon request.

Staining procedure

Refer to the following table for conditions specifically recommended for this antibody. Also refer to detection system data sheets for guidance on specific staining protocols or other requirements.

<u>Parameters</u> <u>Nordic BioSites recommendations</u>

*Pre-treatment Heat Induced Epitope Retrieval (for example in Citrate Buffer pH 6.0 (BCB-

20015/-20016)

*Control tissue Lymphatic vessels (i.e. colon)
*Working dilution 1:50-1:100 (for concentrates)

*Incubation time 30-60 minutes

The antibody is also suitable for Western Blot.

Quality control

The recommended positive control tissue for this antibody is an intestinal tissue including lymphatic vessels. We recommend carrying out a positive and a negative control with every staining run. Please refer to the instructions of the detection system for guidance on general quality control procedures.

Troubleshooting

If you observe unusual staining or other deviations from the expected results please read these instructions carefully, refer to the instructions of the detection system for relevant information or contact your local distributor.

Expected results

This antibody stains positive in the cytoplasm of epithelial cells of lymphatic vessels in formalin-fixed, paraffinembedded tissue sections. Interpretation of the staining results is solely the responsibility of the user. Any experimental result should be confirmed by a medically established diagnostic procedure.

Technical note: Lymphatic vessel endothelium is often only a very small line. Therefore it could be useful to prepare tissue sections of about 2-3

um thickness.

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Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983). Endogenous peroxidase, alkaline phosphatase or biotin may cause non-specific staining depending on the detection system used. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection systems (Omata et al, 1980). Inadequate counterstaining and mounting can influence the interpretation of the results.

Nordic Biosite warrants that the product will meet all requirements described from its shipping date until the expiry date is reached, if the product is stored and utilised as recommended. No additional guarantees can be given. Under no circumstances shall Nordic Biosite be liable for any damages arising out of the use of the reagent provided.

Performance characteristics

Nordic Biosite has conducted studies to evaluate the performance of the antibody for use with a standard detection system. The product has been found to be sensitive and specific to the antigen of interest with minimal or no cross-reactivity.

Bibliography

Marks A et al. Br J Cancer 80:569 pp, 1999 Kahn HJ and Marks A. Lab Invest 82:1255 pp, 2002 Fogt F et al. Oncol Rep 11:47-50, 2004 Ordonez NG. Hum Pathol 36:372-380, 2005 Chu AY et al. Mod Pathol 18:105-110, 2005 Franke FE et al. J Cutan Pathol 31:362-367, 2004 Schacht V et al. Am J Pathol 166:913-921, 2005 Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983 Omata M et al. Am J Clin Pathol 73(5): 626-32, 1980

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