



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

Cat. No.: MSK057 (1 ml Concentrate); MSK057-05 (0.5 ml Concentrate);
MSG057 (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 IgG1
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. MSK057)
Concentrate:	0.5 ml	(Cat. No. MSK057-05)
Ready-to-use:	6 ml	(Cat. No. MSG057)

Dilution of primary antibody

Dilution of Zytomed Systems' 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 Zytomed Systems' recommendations see chapter 'Staining procedure'.

Storage and handling

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

Dilutions of the concentrated antibody should be done with a suitable antibody dilution buffer (e.g. ZUC025 from Zytomed Systems). 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 Zytomed Systems' 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>Zytomed Systems recommendations</u>
*Pre-treatment	Heat Induced Epitope Retrieval (for example in Citrate Buffer pH 6.0 ZUC028)
*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, paraffin-embedded 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 µm thickness.

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.

Zytomed Systems 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 Zytomed System be liable for any damages arising out of the use of the reagent provided.

Performance characteristics

Zytomed Systems 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

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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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Explanations of the symbols on the product label:

REF	Bestellnummer Catalog Number Référence du catalogue		Verwendbar bis Use By Utiliser jusque		Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation
LOT	Chargenbezeichnung Batch Code Code du lot		Lagerungstemperatur Temperature Limitation Limites de température	RUO	Nur für Forschungszwecke For Research Use Only Pour la recherche uniquement
IVD	In vitro Diagnostikum In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro		Achtung Warning Attention		Hersteller / Manufacturer / Fabricant Zytomed Systems GmbH • Anhaltinerstraße 16 14163 Berlin, Germany • Tel: (+49) 30-804 984 990 www.zytomed-systems.com