FLEX Monoclonal Mouse Anti-Human Melanosome Clone HMB45 Ready-to-Use (Dako Autostainer/Autostainer Plus)

Code IS052

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human Melanosome, Clone HMB45, Ready-to-Use (Dako Autostainer/Autostainer Plus), is intended for use in immunohistochemistry (IHC) together with Dako Autostainer/Autostainer Plus instruments. This antibody is useful for the identification of melanocytes with immature melanosome formation in normal skin and nevus. Results aid in the classification of melanomas and melanocytic lesions (1, 3, 5, 6). Differential classification is aided by the results from a panel of antibodies. 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. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Summary and explanation

The presence of the antigen indicates active melanosome formation and thus melanocytic differentiation (5). It is also expressed in normal fetal melanocytes (2,3), but not in normal resting adult melanocytes, regardless of the degree of pigmentation (1,3,6). Upon activation, adult melanocytes can re-express the HMB45-defined antigen (as it is expressed in fetal melanocytes). Such melanocytes are activated by a variety of stimuli. For example, HMB45-labeled cells have been detected in tissue overlying or adjacent to granulation tissue, hemangiomas, vessel-rich tumor stroma, and basal cell carcinoma (5-8). Hair follicles stain occasionally due to associated stimulated melanocytes (1). HMB45 staining has not been observed with melanocytes in lentigines or overlying fibroblastic proliferations such as keloids, dermatofibromas and old fibrotic hemangiomas (8). Non-melanocytic normal tissues do not seem to react with the HMB45 antibody.

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

Reagent provided

Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L NaN_3.

Clone: HMB45. Isotype: IgG1, kappa.

Immunogen

Extract of pigmented melanoma metastases from lymph nodes.

Specificity

Anti-melanosome, HMB45 has been shown to react with a 10 kDa segment of a neuraminidase-sensitive sialylated glycoconjugate present in pre- and early-stage (immature) melanosomes (2-4).

Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation

The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment with heat-induced epitope retrieval (HIER) is required. Optimal results are obtained by pretreating tissues using EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004).

Deparaffinized sections: Pre-treatment of deparaffinized formalin-fixed, paraffin-embedded tissue sections is recommended using Dako PT Link. For details, please refer to the PT Link User Guide. The following parameters should be used for PT Link: Pre-heat temperature: 65 °C; epitope retrieval temperature and time: 97 °C for 20 (±1) minutes; cool down to 65 °C. Rinse sections with diluted room temperature EnVision FLEX Wash Buffer (20x) (Code K8007).

Paraffin-embedded sections: As alternative specimen preparation, both deparaffinization and epitope retrieval can be performed in the PT Link using a modified procedure. See the PT Link User Guide for instructions. After the staining procedure has been completed, the sections must be dehydrated, cleared and mounted using a permanent mounting method.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of Dako Silanized Slides (Code S3003) is recommended.

Staining procedure

The recommended visualization system is EnVision FLEX, High pH (Dako Autostainer/Autostainer Plus) (Code K8010). The staining steps and incubation times are pre-programmed into the software of Dako Autostainer/Autostainer Plus instruments, using the following protocols:

Template protocol: FLEXRTU2 (200 uL dispense volume) or FLEXRTU3 (300 uL dispense volume)

Autoprogram (without counterstaining): HMB45 or Autoprogram (with counterstaining): HMB45H

The Auxiliary step should be set to “rinse buffer” in staining runs with ≤10 slides. For staining runs with >10 slides the Auxiliary step should be set to “none.” This ascertains comparable wash times.

All incubation steps should be performed at room temperature. For details, please refer to the Operator’s Manual for the dedicated instrument. If the protocols are not available on the used Dako Autostainer instrument, please contact Dako Technical Support.

Optimal conditions may vary depending on specimen and preparation methods, and should be determined by each individual laboratory.

Counterstaining in hematoxylin is recommended using EnVision FLEX Hematoxylin (Dako Autostainer/Autostainer Plus) (Code K8018).

Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include melanoma cells and the cells/structures should display reaction patterns as described for this tissue in “Performance characteristics”. The recommended negative control reagent is FLEX Negative Control, Mouse (Dako Autostainer/Autostainer Plus) (Code IS750).

Staining interpretation

The cellular staining pattern is cytoplasmic.

Performance characteristics

Normal tissues: Normal adult tissues that exhibit positive staining with anti-melanosome, HMB45 include melanocytes (fetal and subset, melanocytes containing immature melanosomes), retinal pigment epithelia (prenatal and infantile). Negative tissues include adrenal gland, brain, breast, gallbladder, gastrointestinal tract, kidney, liver, lung, lymphoid tissue, mesenchymal cells, pancreas, peripheral nervous tissue, retinal pigment epithelia (adult), salivary gland, skin (melanocytes, normal resting, melanophages, Langerhans cells, keratinocytes, hair follicles, cutaneous nerves, sweat glands, sebaceous) and testis.

Abnormal tissues: Anti-melanosome, HMB45 stains 245/256 (95.7%) of melanoma (excluding desmoplastic) (1,3,5,6,9-14) and 245/291 (84.2%) of melanoma (including desmoplastic) (1,3,5,6,9,13-14). Melanocytic atypical hyperplasia (2/2) (1), melanocytic neuroectoderm of infancy (1/1) (6), renal angiomyolipoma (27/29) (11,12) and various nevi (218/228) (1,5,6,10) are stained by anti-melanoma, HMB45.

References

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Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code		Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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