INTENDED USE:
The Monoclonal Mouse Anti-Human Melanosome, Clone HMB45 (anti-
melanosome, HMB45) is intended for laboratory use to identify
qualitatively by light microscopy melanocytes with immature
melanosome formation in normal and pathological paraffin-embedded
tissue using immunohistochemical (IHC) test methods. Positive results
aid in the classification of melanomas and melanocytic lesions and also
aid in distinguishing metastatic amelanotic melanomas from other poorly
differentiating tumors of uncertain origin. Differential identification is
aided by the results from a panel of antibodies. Interpretation must be
made within the context of the patient’s clinical history and other
diagnostic tests by a qualified pathologist.

MATERIALS REQUIRED, NOT SUPPLIED:
Refer to the “General Instructions for Immunohistochemical
Staining” or the Detection System “Instructions” of IHC procedures
for:
1. Principle of Procedure, (2) Materials Required, Not Supplied,
(3) Staining Procedure, (4) Quality Control, (5) Troubleshooting,
(6) Interpretation of Staining, (7) General Limitations

SUMMARY AND EXPLANATION:
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 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-positive 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.9 Positive
HMB45 staining has not been observed with melanocytes in lentigines
or overlying fibroblastic proliferations such as keloids, dermatofibromas
and old fibrotic hemangiomas.9 Non-melanocytic normal tissues do not
react with the HMB45 antibody.

REAGENTS PROVIDED:
The primary antibody is available in 7 mL or 22 mL volumes as mouse
antibody in tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.6,
containing stabilizing protein and 0.015 mol/L sodium azide.

The negative control reagent is available in 5 mL or 11 mL volumes as
fetal calf serum in 0.05 mol/L Tris-HCl, pH 7.6, containing stabilizing
protein and 0.015 mol/L sodium azide.

STAINING PROCEDURE:
Follow the procedure for the detection system selected. Use the
recommended incubation time presented in the “Reagents Provided”
section above.
PRODUCT SPECIFIC LIMITATIONS:

1. An HMB45-negative result does not exclude melanoma. Desmoplastic melanomas infrequently stain with the anti-melanosome, HMB45 antibody.\[^{11}\] Therefore it is recommended that this antibody be used in a panel of antibodies including anti-S-100. Anti-S-100 stains desmoplastic melanomas, but lacks the specificity of anti-melanosome, HMB45 for melanomas.

2. Anti-melanosome, HMB45 reacts with a melanocytic antigen, not a melanoma antigen. Anti-melanosome, HMB45 reactivity cannot differentiate between benign and malignant melanocytic lesions. Malignancy must be determined by other criteria.

3. Stimulated normal adult melanocytes (i.e. melanocytes overlying or adjacent to granulation tissue, hemangiomas, vessel-rich stroma, and basal cell carcinomas) may express melanocytic antigens.\[^{5,6,9}\] In some basal cell carcinomas, rare HMB45-positive cells with dendritic morphology are scattered throughout and adjacent to the tumor. The origin of these HMB45-positive cells is unclear, and may represent a "reactive" melanocytic population within the tumor.\[^{1}\]

4. Primary renal neoplasms are nonreactive, except for renal angiomyolipoma (27/29)\[^{13,14}\] and various nevi (218/228)\[^{1,5,6}\].

5. Smooth muscle cells of pulmonary lymphangiomyomatosis (PLAM) exhibit an unusual phenotype characterized by anti-melanosome, HMB45 immunoreactivity distinct from other smooth muscle cell proliferations. Bonetti et al.\[^{14}\], tested 75 pathological lung tranbronchial biopsies and only 6 PLAM specimens from 3 patients showed the presence of HMB45-positive cells.

6. Rare, false-positive staining has been reported for nonmelanocytic tumors such as adenocarcinoma,\[^{15,16}\] olfactory neuroblastoma,\[^{15}\] malignant lymphoma,\[^{15}\] clear cell tumor of the lung,\[^{15}\] and plasmacytoma\[^{16}\] with anti-melanosome, HMB45 ascites preparations.\[^{15,17}\] Nonspecific staining of normal breast epithelia, sweat glands, plasma cells and bronchial epithelia has also been observed with ascites-derived antibodies and appears as membranous and/or granular, cytoplasmic staining.\[^{15,17}\] These artifacts have been attributed to contaminated ascites preparation or inappropriately diluted antibody.\[^{4,15-17}\] No such staining has been reported with tissue culture supernatant preparations. Anti-melanosome, HMB45 is produced from tissue culture supernatant. If false-positive staining of nonmelanocytic tumors or normal epithelia occurs with anti-melanosome, HMB45, report the event to DAKO’s Technical Service Department.

7. This antibody does not work in Western blot assays.\[^{1}\]

PERFORMANCE CHARACTERISTICS:

Reproducibility: The cellular staining pattern for anti-melanosome, HMB45 is cytoplasmic. Anti-melanosome, HMB45 has been tested on serial sections of tissue specimens. Consistent staining results have been obtained with run-to-run and within run antibody testing.

Normal tissues: Normal adult tissue that exhibit positive staining with anti-melanosome, HMB45 include melanocytes (fetal and subset, melanocytes containing immature melanosomes), retinal pigment epithelia (prenatal and infantile). Negative tissue include adrenal gland, brain, breast, gallbladder, gastrointestinal tract, kidney, liver, lung, lymphoid tissue, mesenchymal and mesothelial cells, pancreas, peripheral nervous tissue, retinal pigment epithelium (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)\[^{1,3,5,8,14,18-20}\] and 245/291 (84.2%) of melanoma (including desmoplastic)\[^{1,3,5,8,11,18-20}\]. Melanocytic atypical hyperplasia (2/2),\[^{1}\] melanocytic neuroectoderm of infancy (1/1),\[^{8}\] renal angiomyolipoma (27/29)\[^{13,14}\] and various nevi (218/228)\[^{7,5,8,12}\] are stained by anti-melanoma, HMB45.

REFERENCES:

20. Herman GE. Unpublished data. DAKO Corp. 1992

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