Product name: Anti-Melanoma antibody [HMB45 + M2-7C10 + M2-9E3 + T311] ab733

Description: Mouse monoclonal [HMB45 + M2-7C10 + M2-9E3 + T311] to Melanoma

Host species: Mouse

Specificity: The HMB45 clone reacts with a neuraminidase-sensitive oligosaccharide side chain of a glycoconjugate present in immature melanosomes. The HMB45-reactive antigen is present in cutaneous melanocytes, prenatal and infantile retinal pigment epithelium and melanoma cells and is thought to be oncofetal in nature. This antibody has been shown to label the majority of melanomas. MART-1 recognizes a protein of 18kDa, identified at MART-1 (Melanoma Antigen Recognized by T cells 1) or Melan-A. Melan-A is a useful addition to melanoma panels as it is apparently specific for melanocytic lesions. Studies have also shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas. Tyrosinase is a key enzyme involved in the initial stages of melanin biosynthesis. Studies have shown Tyrosinase to be a more sensitive marker when compared to HMB45 and MART-1. It has also shown to label a higher percentage of desmoplastic melanomas than HMB45.

Tested applications: Suitable for: Flow Cyt, ICC/IF, IHC-P, IHC-Fr

Species reactivity: Reacts with: Human

Immunogen: HMB45 - Pigmented melanoma metastases from LN MART-1 - Recombinant human MART-1 protein Tyrosinase - Recombinant tyrosinase protein

Positive control: Metastatic melanoma in lymph node.

General notes: Please note that this antibody is an oligoclonal antibody. It is a cocktail of monoclonal antibodies that have been carefully selected. Oligoclonal antibodies have not only the specificity and batch-to-batch consistency of a monoclonal antibody, but also have the advantage of the sensitivity of a polyclonal antibody due to their ability to recognize multiple epitopes on an antigen.

The combination of HMB45, MART-1 (M2-7C10 + M2-9E3) and Tyrosinase (T311) make this quadruple antibody cocktail a first-order pan melanoma screener, and may prove to be a valuable marker for melanoma metastasis in sentinel lymph nodes (see reference 3.).

Properties

Form: Liquid

Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C or -80°C. Avoid freeze /
thaw cycle.

**Storage buffer**
Preservative: Proprietary, not sodium azide or thimerosal.
Constituents: PBS, Tissue Culture Supernatant. pH 7.3; Protein carrier.

**Primary antibody notes**
The combination of HMB45, MART-1 (DT101 + BC199) and Tyrosinase (T311) make this quadruple antibody cocktail a first-order pan melanoma screener, and may prove to be a valuable marker for melanoma metastasis in sentinel lymph nodes (see reference 3.).

**Clonality**
Monoclonal

**Clone number**
HMB45 + M2-7C10 + M2-9E3 + T311

**Myeloma**
Unknown

**Isotype**
IgG

**Light chain type**
Kappa

### Applications

Our [Abpromise guarantee](#) covers the use of [ab733](#) in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<th>Abreviews</th>
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<td>Flow Cyt</td>
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<td>1/100.</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-P</td>
<td>1/25 - 1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. It is sometimes difficult to interpret DAB stained melanomas due to endogenous pigment, therefore we recommend you substitute an AEC or a Fast Red substrate protocol.</td>
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<tr>
<td>IHC-Fr</td>
<td>1/25 - 1/50. ABC method.</td>
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### Target

**Relevance**
Malignant melanoma is a malignant neoplasm of melanocytes, arising de novo or from a pre-existing benign nevus, which occurs most often in the skin but also may involve other sites.

**Cellular localization**
Membrane; Single-pass membrane protein

**Images**
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Melanoma antibody [HMB45 + M2-7C10 + M2-9E3 + T311] (ab733)

IHC image of ab733 staining in human melanoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab733, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

ab733 staining Melanoma by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).
Flow Cytometry - Anti-Melanoma antibody [HMB45 + M2-7C10 + M2-9E3 + T311] (ab733)

Overlay histogram showing MALME 3M cells stained with ab733 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab733, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG (1μg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Immunofluorescence analysis of cultured Human melanoma cells, staining Melanoma with ab733.

Cells were fixed with formaldehyde and blocked with blocking reagent for 30 minutes at room temperature. Samples were incubated with primary antibody (1/25 in blocking solution) for 1 hour at 37°C. An AlexaFluor®594-conjugated donkey anti-mouse polyclonal IgG (1/200) was used as the secondary antibody.

Immunocytochemistry/ Immunofluorescence - Anti-Melanoma antibody [HMB45 + M2-7C10 + M2-9E3 + T311] (ab733)

This image is courtesy of an Abreview submitted by Hongwei Shao

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