abcam

Product datasheet

Anti-MelanA antibody [EPR20380] ab210546

Recombinant RabMAb

★★★★★ 15 Abreviews 22 References 10 Images

Overview

Product name Anti-MelanA antibody [EPR20380]

Description Rabbit monoclonal [EPR20380] to MelanA

Host species Rabbit

Tested applications Suitable for: IHC-P, WB, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human melanoma lysate; B16F0 and MeWo whole cell lysates. IHC-P: Human malignant

melanoma and metastatic malignant melanoma tissues; Mouse hair bulb tissue. ICC/IF: MeWo

and B16F0 cells. Flow Cyt (intra): B16F0 cells, Malme-3M cells.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR20380

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab210546 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	****(8)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	****(1)	1/2000. Detects a band of approximately 18 kDa (predicted molecular weight: 13 kDa).
ICC/IF	★★★★★ (1)	1/1000.
Flow Cyt (Intra)		1/500. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

Target

Function

Involved in melanosome biogenesis by ensuring the stability of GPR143. Plays a vital role in the expression, stability, trafficking, and processing of melanocyte protein PMEL, which is critical to the formation of stage II melanosomes.

Expression is restricted to melanoma and melanocyte cell lines and retina.

Tissue specificity

Post-translational Acv

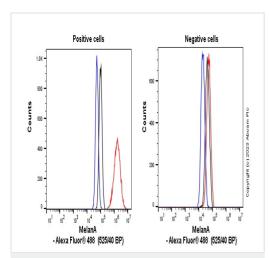
modifications

Acylated.

Cellular localization

Endoplasmic reticulum membrane. Golgi apparatus. Golgi apparatus > trans-Golgi network membrane. Melanosome. Also found in small vesicles and tubules dispersed over the entire cytoplasm. A small fraction of the protein is inserted into the membrane in an inverted orientation. Inversion of membrane topology results in the relocalization of the protein from a predominant Golgi/post-Golgi area to the endoplasmic reticulum. Melanoma cells expressing the protein with an inverted membrane topology are more effectively recognized by specific cytolytic T-lymphocytes than those expressing the protein in its native membrane orientation.

Images



Flow Cytometry (Intracellular) - Anti-MelanA antibody [EPR20380] (ab210546)

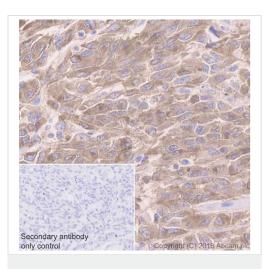
Flow cytometry overlay histogram showing left Malme-3M positive cells and right negative HEK293 stained with ab210546 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1 % PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab210546) (1x 10^6 in 100μ l at 0.2μ g/ml (1/10500)) for 30min at 22° C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C Isotype control antibody (black line) was Recombinant Rabbit IgG,

monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Malme-3M Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1 % PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MelanA antibody
[EPR20380] (ab210546)

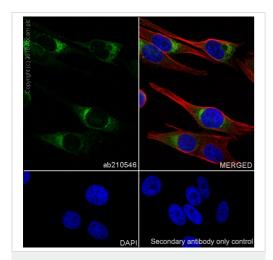
Immunohistochemical analysis of paraffin-embedded human malignant melanoma tissue labeling MelanA with ab210546 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on human malignant melanoma is observed [PMID: 17445277] [PMID: 21840568].

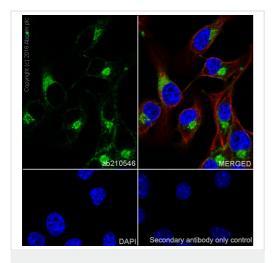
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-MelanA antibody [EPR20380] (ab210546)



Immunocytochemistry/ Immunofluorescence - Anti-MelanA antibody [EPR20380] (ab210546)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MeWo (Human malignant melanoma cell line) cells labeling MelanA with ab210546 at 1/1000 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on MeWo cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab195889</u> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.

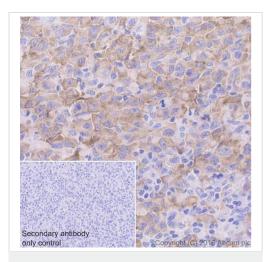
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized B16F0 (Mouse melanoma cell line) cells labeling MelanA with ab210546 at 1/1000 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on B16F0 cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab195889</u> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MelanA antibody
[EPR20380] (ab210546)

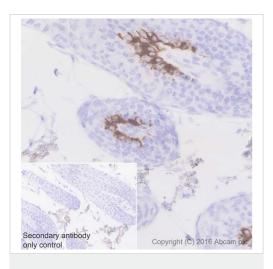
Immunohistochemical analysis of paraffin-embedded human metastatic malignant melanoma tissue labeling MelanA with ab210546 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on human metastatic malignant melanoma is observed [PMID: 17445277] [PMID: 21840568].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MelanA antibody
[EPR20380] (ab210546)

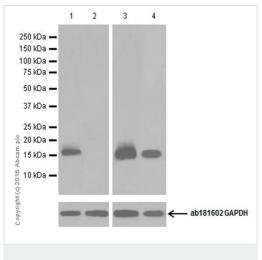
Immunohistochemical analysis of paraffin-embedded mouse hair bulb tissue labeling MelanA with ab210546 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic staining on melanocytes of mouse hair bulb is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-MelanA antibody [EPR20380] (ab210546)

All lanes : Anti-MelanA antibody [EPR20380] (ab210546) at 1/2000 dilution

Lane 1: Human melanoma lysate

Lane 2 : A-375 (Human malignant melanoma cell line) whole cell

Lane 3: B16F0 (Mouse melanoma cell line) whole cell lysate

Lane 4 : MeWo (Human malignant melanoma cell line) whole cell

lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 13 kDa **Observed band size:** 18 kDa

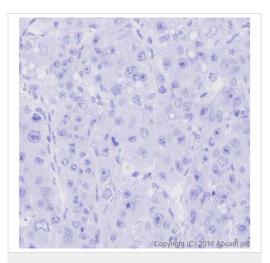
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 10 seconds; Lane 3 and 4: 3 seconds.

A-375 doesn't express MelanA. The expression profile observed is consistent with what has been described in the literature (PMID: 12670905).

MelanA – Alexa Fluor® 488 (530/30 BP)

Flow Cytometry (Intracellular) - Anti-MelanA antibody [EPR20380] (ab210546) Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed B16F0 (Mouse melanoma cell line) cells labeling MelanA with ab210546 at 1/500 dilution (red) compared with a rabbit monoclonal lgG isotype control (ab172730; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



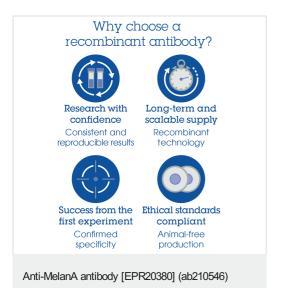
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MelanA antibody
[EPR20380] (ab210546)

Immunohistochemical analysis of paraffin-embedded human liver cancer tissue labeling MelanA with ab210546 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Negative control: no staining on human liver cancer [PMID: 10096358].

Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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