abcam

ab256497

Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP4863(2)
Purification technique	Affinity purification Protein A
Concentration	0.975 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Human
Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information PMEL

Function	Forms physiological amyloids that play a central role in melanosome morphogenesis and pigmentation. The maturation of unpigmented premelanosomes from stage I to II is marked by assembly of processed amyloidogenic fragments into parallel fibrillar sheets, which elongate the vesicle into a striated ellipsoidal shape. In pigmented stage III and IV melanosomes, the amyloid matrix serves as a platform where eumelanin precursors accumulate at high local concentrations for pigment formation. May prevent pigmentation-associated toxicity by sequestering toxic reaction intermediates of eumelanin biosynthesis pathway. Represents a potent melanoma-specific antigen. Among melanoma non-mutated self-peptides, G9-154 (KTWGQYWQV), G9-209 (ITDQVPFSV)

and G9-280 (YLEPGPVTA), appear to act as immunodominant common epitopes that stimulate anti-tumor immune response mediated by HLA-A-restricted cytotoxic T cells.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab256497 is the carrier-free version of ab137078.

Patented technology

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

Species reactivity

Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

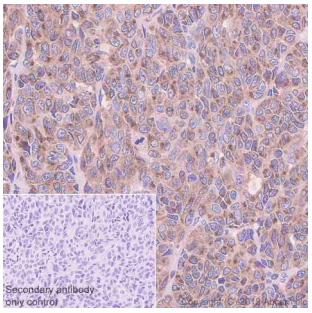
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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8 product images

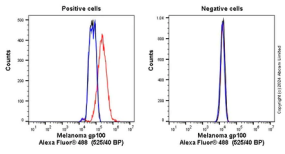


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

Melanoma gp100 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Melanoma gp100 antibody

ab137078 staining Melanoma gp100 in human melanoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250 for 30 minutes at room temperature. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody.
The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab137078).



Flow Cytometry (Intracellular) - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

Melanoma gp100 Flow Cytometry (Intracellular) staining using rabbit Anti-Melanoma gp100 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab137078).

Flow cytometry overlay histogram showing left B16-F10 positive cells and right negative Raw264.7 stained with ab137078 (red line). The cells were fixed with 80% methanol (5 mins) and then

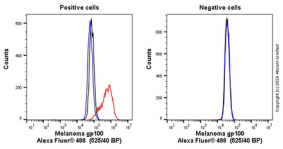
permeabilised with 0.1% PBS-Triton X-100 for 15 mins. The cells were then incubated in 1x PBS containing 10µg/ml anti CD16/CD32 and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab137078) (1x 10^6 in 100µl at 1.0µg/ml (1/500 dilution)) for 30 mins at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 dilution for 30 mins at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) 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 B16-F10 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 minss under the same conditions.



Flow Cytometry (Intracellular) - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

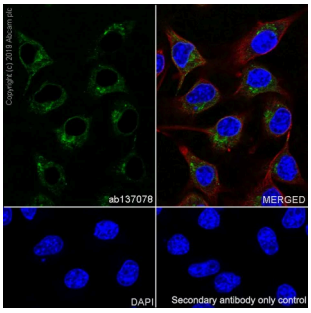
Melanoma gp100 Flow Cytometry (Intracellular) staining using rabbit Anti-Melanoma gp100 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab137078).
Flow cytometry overlay histogram showing left Malme-3M positive cells and right negative A-375 stained with ab137078 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 mins. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab137078) (1x 10^6 in 100µl at 1.0µg/ml (1/500 dilution)) for 30 mins at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 dilution for 30 mins at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

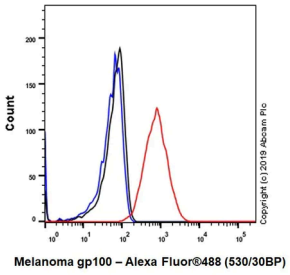
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

Melanoma gp100 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Melanoma gp100 antibody

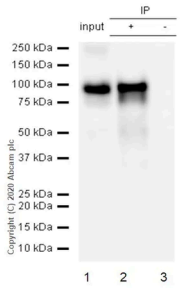
ab137078 staining Melanoma gp100 in B16-F0 (mouse skin) cells by ICC (Immunocytochemistry). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/50. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) ab195889 was used as a counterstain at a dilution of 1/200. DAPI was used as a nuclear counterstain.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab137078).



Flow Cytometry (Intracellular) - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

Melanoma gp100 Flow Cytometry (Intracellular) staining using rabbit Anti-Melanoma gp100 antibody

Overlay histogram showing B16-F0 (Mouse skin melanoma cell) cells stained with ab137078 (red line). The cells were fixed with 4% paraformaldehydeand then permeabilized with 90% methanol. The cells were then incubated with ab137078 at 1/40 dilution. The secondary antibody used wasGoat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution. Isotype control antibody (black line) was Rabbit monoclonal IgG (ab172730). Unlabelled control (blue line) was cells without incubation with primary antibody and secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab137078).



Immunoprecipitation - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

ab137078 immunoprecipitating Melanoma gp100. 10µg of cell lysate was incubated with primary antibody at a dilution of 1/500 and VeriBlot for IP Detection Reagent (HRP) (ab131366) at a dilution of 1/10000.
Lane 1: B16-F0 (mouse melanoma epithelial cell-like) whole cell lysate 10ug
Lane 2: B16-F0 (mouse melanoma epithelial cell-like) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab137078 in B16-F0 whole cell lysate

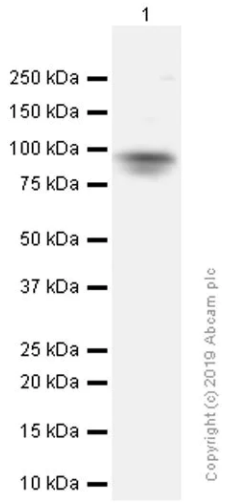
Exposure time: 1 second

Observed MW: 85-100 KDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab137078).

All lanes:
Immunoprecipitation - Anti-Melanoma gp100 antibody [EP4863(2)] (ab137078)

Predicted band size: 70 kDa



Western blot - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

Melanoma gp100 Western blot staining of Human melanoma lysate 15µg using rabbit Anti-Melanoma gp100 antibody

Exposure time: 18-0 seconds
Observed MW: 85-100 KDa

The molecular weights observed are consistent with what have been described in PMID: 16492709 and PMID: 7961886

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab137078).

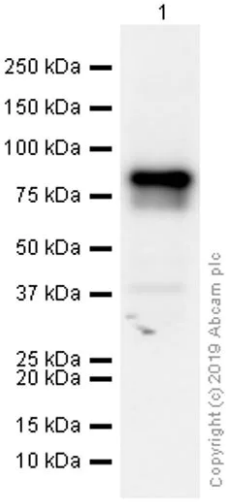
All lanes:
Western blot - Anti-Melanoma gp100 antibody [EP4863(2)] (ab137078) at 1/1000 dilution

All lanes:
Human melanoma lysate 15µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 70 kDa



Western blot - Anti-Melanoma gp100 antibody [EP4863(2)] - BSA and Azide free (ab256497)

Exposure time: 50 seconds
Observed MW: 85-100 KDa

The molecular weights observed are consistent with what have been described in PMID: 16492709 and PMID: 7961886

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab137078).

All lanes:
Western blot - Anti-Melanoma gp100 antibody [EP4863(2)] (ab137078) at 1/10000 dilution

All lanes:
B16-F0 (Mouse melanoma epithelial cell-like) whole cell lysate at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 70 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.