abcam

Product datasheet

Anti-CD147 antibody [EPR18008-8] ab188190

Recombinant RabMAb

11 References 10 Images

Overview

Product name Anti-CD147 antibody [EPR18008-8]

Description Rabbit monoclonal [EPR18008-8] to CD147

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt, IP, Sandwich ELISA

Species reactivity Reacts with: Mouse, Recombinant fragment

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse CD147 recombinant protein fragment; RAW 264.7, WEHI-3 and bEND.3 whole cell

> lysates; mouse liver lysate. IHC-P: Mouse intestine tissue. ICC/IF: WEHI-231 and bEND.3 cells. Flow Cyt: Mouse thymocytes, C57 BL/6 mouse thymocytes. IP: RAW 264.7 whole cell lysate.

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 EPR18008-8

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab188190 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
WB		1/5000. Detects a band of approximately 55 kDa (predicted molecular weight: 42 kDa).
IHC-P		1/8000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/250.
Flow Cyt		1/200. ab172730 - Rabbit monoclonal lgG, is suitable for use an isotype control with this antibody.
IP		1/50.
Sandwich ELISA		Use at an assay dependent concentration.

T	ar	g	e	t

Function Plays pivotal roles in spermatogenesis, embryo implantation, neural network formation and tumor

progression. Stimulates adjacent fibroblasts to produce matrix metalloproteinases (MMPS). May target monocarboxylate transporters SLC16A1, SLC16A3 and SLC16A8 to plasma membranes of retinal pigment epithelium and neural retina. Seems to be a receptor for oligomannosidic

glycans. In vitro, promotes outgrowth of astrocytic processes.

Tissue specificity Present only in vascular endothelium in non-neoplastic regions of the brain, whereas it is present

in tumor cells but not in proliferating blood vessels in malignant gliomas.

Sequence similarities Contains 1 lg-like C2-type (immunoglobulin-like) domain.

Contains 1 lg-like V-type (immunoglobulin-like) domain.

Post-translational

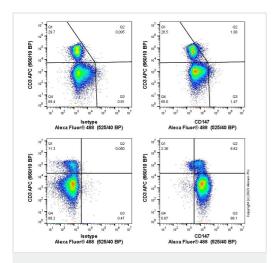
modifications

N-glycosylated.

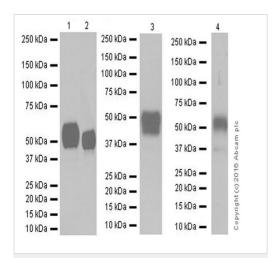
Cellular localization Cell membrane. Melanosome. Colocalizes with SLC16A1 and SLC16A8 (By similarity). Identified

by mass spectrometry in melanosome fractions from stage I to stage IV.

Images



Flow Cytometry - Anti-CD147 antibody [EPR18008-8] (ab188190)



Western blot - Anti-CD147 antibody [EPR18008-8] (ab188190)

Flow cytometry staining of C57 BL/6 mouse splenocytes (top) or C57 BL/6 mouse thymocytes (bottom), with ab188190 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Splenocytes or thymocytes were incubated for 30 min at 4°C in 1x PBS containing 10 μ g/ml anti CD16/CD32 and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by ab188190 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10⁶ in 100 μ l at 0.1 μ g/ml (1/21900)) for 30 min at 4°C. The cells were simultaneously stained with CD3.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 4°C Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.

All lanes : Anti-CD147 antibody [EPR18008-8] (ab188190) at 1/5000 dilution

Lane 1 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lane 2: Mouse liver lysate

Lane 3: WEHI-3 (Mouse leukemia cell line) whole cell lysate

Lane 4: bEnd.3 (Mouse brain endothelioma cell line) whole cell

lysate

Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 42 kDa

Observed band size: 45-55 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1/2: 3 minutes; Lane 3: 10 seconds; Lane 4: 1 second.

The expression profile observed is consistent with what has been described in the literature (PMID: 16721788; 23966157).

ab188190 MERGED

DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-CD147 antibody [EPR18008-8] (ab188190)

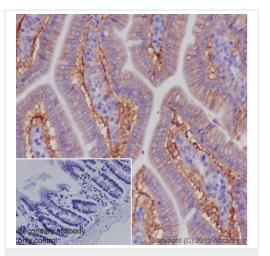
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized bEND.3 (Mouse brain capillary endothelial cell line) cells labeling CD147 with ab188190 at 1/250 dilution, followed by by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cell membrane staining on bEND.3 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (<u>ab150120</u>) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab188190 at 1/250 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

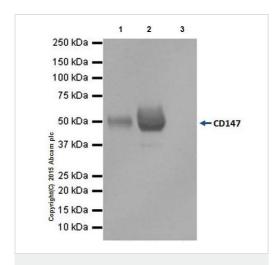


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD147 antibody
[EPR18008-8] (ab188190)

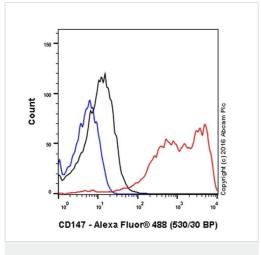
Immunohistochemical analysis of paraffin-embedded mouse intestine tissue labeling CD147 with ab188190 at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membrane staining on mouse intestine is observed. Counter stained with Hematoxylin.

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.



Immunoprecipitation - Anti-CD147 antibody [EPR18008-8] (ab188190)



Flow Cytometry - Anti-CD147 antibody [EPR18008-8] (ab188190)

CD147 was immunoprecipitated from 1 mg of RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate with ab188190 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab188190 at 1/5000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: RAW 264.7 whole cell lysate 10µg (Input).

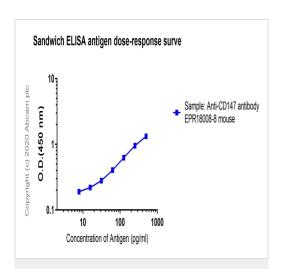
Lane 2: ab188190 IP in RAW 264.7 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G (\underline{ab172730})$ instead of ab188190 in RAW 264.7 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

Flow cytometric analysis of fresh mouse thymocytes labeling CD147 with ab188190 at 1/200 dilution (red) compared with Rabbit lgG, monoclonal [EPR25A] - 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 1/500 dilution was used as the secondary antibody.



Sandwich ELISA - Anti-CD147 antibody [EPR18008-8] (ab188190)

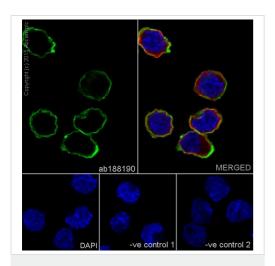
Standard Curve for CD147 (Analyte: Recombinant mouse CD147 protein) dilution range 0-500 pg/mL using Capture antibody at 0.2 ug/mL and Detector Antibody at 0.5 ug/mL. Secondary antibody: Peroxidase Streptavidin SA-HRP at 1/20000 dilution.

Concentration of ab188190 may vary from lot to lot; please use this

Washing buffer: 1X PBST

curve as guideline.

Blocking/Diluting buffer and concentration: 1% BSA/PBS



Immunocytochemistry/ Immunofluorescence - Anti-CD147 antibody [EPR18008-8] (ab188190)

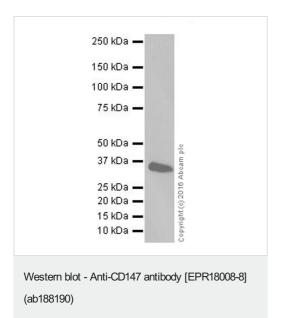
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized WEHI-231 (Mouse B Cell Lymphoma cell line) cells labeling CD147 with ab188190 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cell membrane staining on WEHI-231 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab188190 at 1/250 dilution followed by Goat Anti-Mouse lgG H&L (Alexa Fluor $^{\mbox{\it B}}$ 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Anti-CD147 antibody [EPR18008-8] (ab188190) at 1/5000 dilution

+ Mouse CD147 recombinant protein fragment at 0.002 µg

Secondary

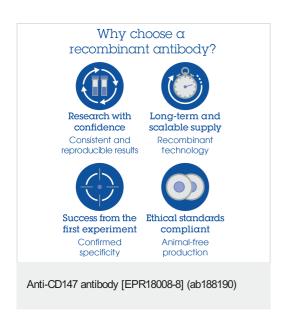
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 42 kDa **Observed band size:** 28 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Recombinant protein fragment Mouse CD147 contains aa140-325 with a His-Tag[®]. It was made in house



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