abcam

Product datasheet

Anti-CD147 antibody [MEM-M6/1] ab666

KO VALIDATED

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Overview

Product name	Anti-CD147 antibody [MEM-M6/1]
Description	Mouse monoclonal [MEM-M6/1] to CD147
Host species	Mouse
Specificity	Human CD147 antigen. This antibody recognizes an epitope in the N-terminal Ig domain (D1). This high-affinity antibody is capable of binding to unstimulated peripheral blood T cells.
Tested applications	**Suitable for:** IHC-P, Flow Cyt, WB, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant full length protein corresponding to Human CD147. Purified soluble recombinant form of CD147, CD147Rg, which consists of the cDNA coding for the hinge region, CH2-and CH3 domain of human IgG1 (CD147Rg is secreted by transfectants as a dimer). Database link: **P35613**
Positive control	This antibody gave a positive result in IHC in the following FFPE tissue: Human normal heart muscle. Flow Cytometry: A549 cells and Peripheral blood lymphocytes. WB: A549, Raji and Jurkat.
General notes	This product was changed from ascites to tissue culture supernatant on 24th January 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.40

	Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Protein A purified
Purification notes	Purified from TCS. Purity >95% by SDS-PAGE.
Clonality	Monoclonal
Clone number	MEM-M6/1
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab666 in the following tested applications.

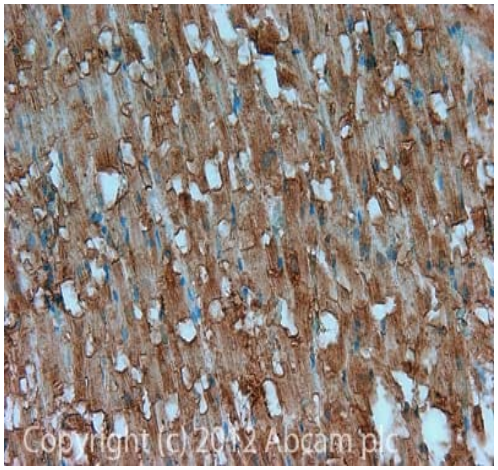
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★☆☆ (3)	Use a concentration of 10 µg/ml.
Flow Cyt		Use a concentration of 10 µg/ml. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (1)	Use a concentration of 1 µg/ml. Use under non reducing condition.
IP		Use at an assay dependent concentration.

Target

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 Ig-like C2-type (immunoglobulin-like) domain. Contains 1 Ig-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.

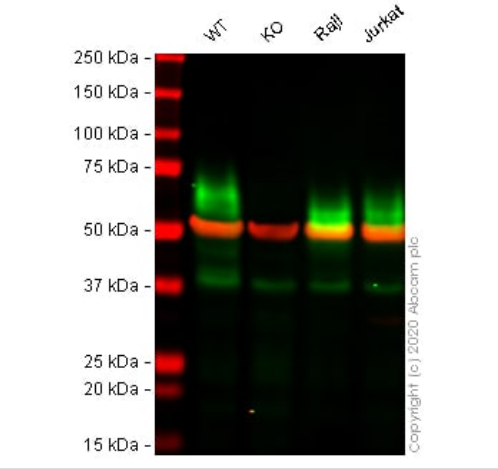
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD147 antibody [MEM-M6/1] (ab666)

IHC image of CD147 staining in human normal heart muscle formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab666, 5 µg/ml, for 15 minutes 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.



Western blot - Anti-CD147 antibody [MEM-M6/1] (ab666)

All lanes : Anti-CD147 antibody [MEM-M6/1] (ab666) at 1 µg/ml

Lane 1 : Wild-type A549 cell lysate
Lane 2 : BSG knockout A549 cell lysate
Lane 3 : Raji cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 30 µg per lane.

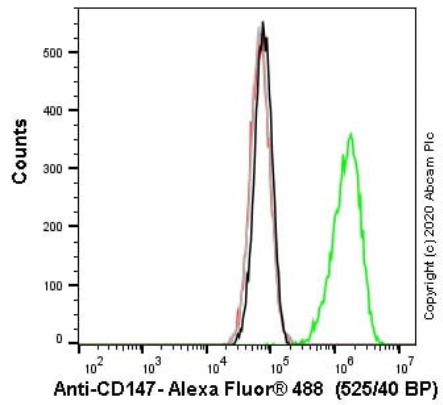
Performed under reducing conditions.

Observed band size: 55-70 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab666 observed at 55-70 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab666 was shown to react with CD147 in wild-type A549 cells in western blot with loss of signal observed in BSG knockout cell line **ab273748** (knockout cell lysate **ab275500**). Wild-type and BSG knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab666 and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed

(**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



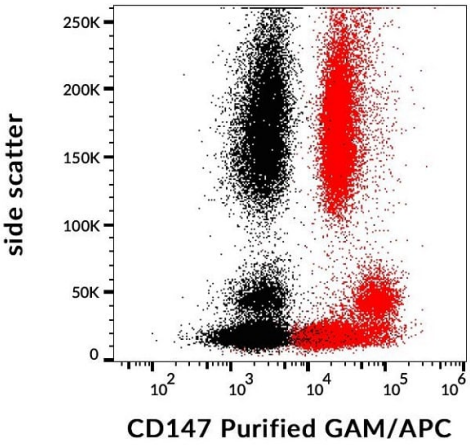
Flow Cytometry - Anti-CD147 antibody [MEM-M6/1] (ab666)

Flow cytometry overlay histogram showing wild-type A549 (green line) and BSG knockout A549 cells (**ab273748**) stained with ab666 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab666) ($1x10^6$ in 100µl at 10 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

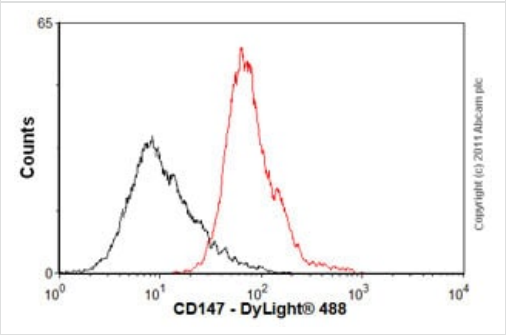
Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type A549 - black line; BSG knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

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



Flow Cytometry - Anti-CD147 antibody [MEM-M6/1] (ab666)

Flow cytometry of human peripheral blood cells with ab666 at 1 µg/ml



Flow Cytometry - Anti-CD147 antibody [MEM-M6/1] (ab666)

Overlay histogram showing peripheral blood lymphocytes stained with ab666 (red line). The cells were incubated with the antibody (ab666, 1 µg/1x10^6 cells) for 30 minutes at 4ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 minutes at 4ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2 µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed gating on peripheral blood lymphocytes.

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