Product datasheet

Anti-CD147 antibody ab64616

Overview

Product name  Anti-CD147 antibody
Description  Rabbit polyclonal to CD147
Host species  Rabbit
Tested applications  Suitable for: WB, IHC-P, IP, ICC/IF
Species reactivity  Reacts with: Mouse, Human
Predicted to work with: Orangutan
Immunogen  Synthetic peptide conjugated to KLH derived from within residues 350 to the C-terminus of Human CD147. Read Abcam's proprietary immunogen policy (Peptide available as ab71911.)
Positive control  Recombinant Human CD147 protein (ab114195) can be used as a positive control in WB. This antibody gave a positive signal in the following Lysates: HeLa Whole Cell - Hydroxyurea Treated (48hr, 2uM), HeLa Whole Cell - Bleomycin Treated (20U/ml), HeLa Whole Cell - Staurosporine Treated (48hr, 500nM), Y79, Mouse Skeletal Muscle Tissue, HeLa, SK N SH Whole Cell This antibody gave a positive result when used in the following methanol fixed cell lines: SV40LT-SMC IHC-P: FFPE human heart tissue sections.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer  Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity  Immunogen affinity purified
Clonality  Polyclonal
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab64616 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 60 kDa (predicted molecular weight: 42 kDa).</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use a concentration of 5 µg/ml.</td>
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<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 5 µg/ml.</td>
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</tbody>
</table>

**Target**

**Function**

**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**

All lanes : Anti-CD147 antibody (ab64616) at 1 µg/ml

Lane 1 : HeLa Whole Cell Lysate - Hydroxyurea Treated (48hr, 2uM)  
Lane 2 : HeLa Whole Cell Lysate - Bleomycin Treated (20U/ml)  
Lane 3 : HeLa Whole Cell Lysate - Staurosporine Treated (48hr, 500nM)  
Lane 4 : Y79 (Human retinoblastoma cell line) Whole Cell Lysate  
Lane 5 : Skeletal Muscle (Mouse) Tissue Lysate  
Lane 6 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate  
Lane 7 : SK N SH (Human neuroblastoma) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.
Secondary

All lanes: Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 42 kDa

Observed band size: 60 kDa

why is the actual band size different from the predicted?

Additional bands at: 150 kDa. We are unsure as to the identity of these extra bands.

CD147 contains known glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.

IHC image of CD147 staining in human heart 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 ab64616, 1µg/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.
ab64616 stained SV40LT-SMC cells. The cells were 100% methanol fixed (5 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab64616 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-rabbit (ab96899) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

CD147 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to CD147 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab64616.


Band: 60kDa; CD147

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