abcam

Product datasheet

Anti-CD63 antibody [KILL150A] ab271286

Recombinant

★★★★ 4 Abreviews 14 References 7 Images

Overview

Product name Anti-CD63 antibody [KILL150A]

Description Mouse monoclonal [KILL150A] to CD63

Host species Mouse

Specificity We recommend <u>ab134045</u> for low expression samples.

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

Species reactivity Reacts with: Human

Immunogen Tissue, cells or virus. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: SK-MEL-28 and HUVEC whole cell lysate. Human lymph node tissue lysate. IHC-P: Human

melanoma tissue. ICC/IF: SK-MEL-28 cells. Flow Cyt: SK-MEL-28

General notes

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact orders@abcam.com.

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.

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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

Purity Protein A purified

Clonality Monoclonal
Clone number KILL150A

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Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab271286 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	★★★★ (2)	Use a concentration of 0.978 µg/ml. Predicted molecular weight: 26 kDa.
IHC-P	**** <u>(1)</u>	Use a concentration of 0.978 µg/ml. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		Use a concentration of 19.56 µg/ml.
ICC/IF		Use a concentration of 19.56 µg/ml.

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Function This antigen is associated with early stages of melanoma tumor progression. May play a role in

growth regulation.

Tissue specificity Dysplastic nevi, radial growth phase primary melanomas, hematopoietic cells, tissue

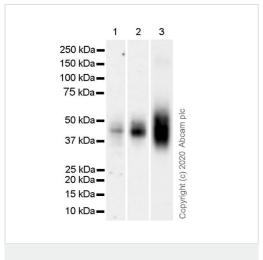
macrophages.

Sequence similarities Belongs to the tetraspanin (TM4SF) family.

Cell membrane. Lysosome membrane. Late endosome membrane. Also found in Weibel-Palade

bodies of endothelial cells. Located in platelet dense granules.

Images



Western blot - Anti-CD63 antibody [KILL150A] (ab271286)

All lanes : Anti-CD63 antibody [KILL150A] (ab271286) at 0.978 $\mu g/ml$

Lane 1 : SK-MEL-28 (human malignant melanoma), whole cell lysate

Lane 2: HUVEC (human umbilical vein endothelial cell), whole cell lysate

Lane 3: Human lymph node tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Predicted band size: 26 kDa

CD63 can undergo glycosylation as shown in lane 1, 2 and 3 (PMID: 9890706, 28740179).

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1-2: 3 mins; Lane 3: 48 secs.

This blot was developed using a higher sensitivity ECL substrate.

Western blot - Anti-CD63 antibody [KILL150A] (ab271286)

All lanes : Anti-CD63 antibody [KILL150A] (ab271286) at 1/1000 dilution

Lanes 1 & 5 : Un-boiled HUVEC (human umbilical vein endothelial cell) whole cell lysate

Lanes 2 & 6: Un-boiled SK-MEL-28 (human malignant melanoma) whole cell lysate

Lane 3 : Boiled HUVEC (human umbilical vein endothelial cell) whole cell lysate

Lane 4: Boiled SK-MEL-28 (human malignant melanoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Developed using the ECL technique.

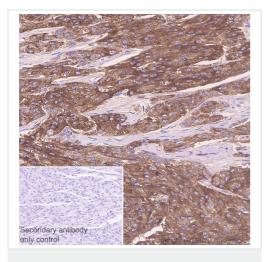
Predicted band size: 26 kDa

Observed band size: 30-60 kDa

Blocking and diluting buffer and concentration: 5% NFDM /TBST

Exposure time: Lane1-4: 180s; Lane 5-6: 80s

We recommend not boiling lysate before loading onto the gel and using a higher sensitive ECL substrate to increase the band intensity.

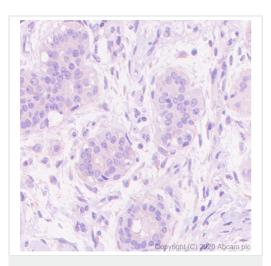


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD63 antibody
[KILL150A] (ab271286)

Immunohistochemical analysis of paraffin-embedded human melanoma tissue labeling CD63 with ab271286 at 1/4000 dilution (0.978µg/ml) followed by ready to use Goat Anti-mouse lgG H&L (HRP polymer) (ab214879). Cytoplasmic staining on human melanoma. The section was incubated with ab271268 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-mouse IgG H&L (HRP polymer) (ab214879).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



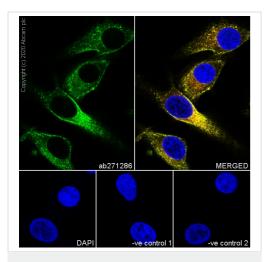
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD63 antibody
[KILL150A] (ab271286)

Immunohistochemical analysis of paraffin-embedded human breast tissue labeling CD63 with ab271286 at 1/4000 dilution (0.978µg/ml) followed by ready to use Goat Anti-mouse IgG H&L (HRP polymer) (ab214879). The section was incubated with ab271268 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Negative control: Nearly no staining on human breast.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-mouse IgG H&L (HRP polymer) (ab214879).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-CD63 antibody [KILL150A] (ab271286)

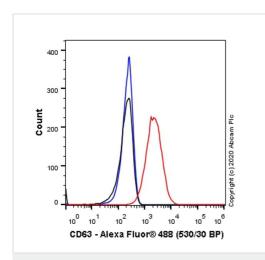
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SK-MEL-28 cells labelling CD63 with ab271286 at 1/50 dilution (19.56µg/ml), followed by **ab150113** Goat Anti-mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing lysosome/endosomes (**ab252919**) co-staining in SK-MEL-28 cells.

<u>ab252919</u> Anti-CD63 rabbit monoclonal antibody was used to counterstain tubulin at 1/250 dilution, followed by <u>ab150080</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 594) at a 1/1000 dilution (Red). The nuclear counterstain was DAPI (Blue).

Negative control 1: ab271286 at a 1/50 dilution (19.56 μ g/ml) followed by ab150080 at a 1/1000 dilution.

Negative control 2: <u>ab259919</u> at a 1/200 dilution followed by ab150113 at a 1/1000 dilution.

Flow cytometric analysis of SK-MEL-28 (human malignant melanoma) cells labelling CD63 with ab271286 at 1/1000 dilution (19.56µg/ml) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.



Flow Cytometry - Anti-CD63 antibody [KILL150A] (ab271286)



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