abcam

Product datasheet

Anti-CD63 antibody [KILL150A] - BSA and Azide free ab271296

`Recombinant`

7 Images

Overview

Product name	Anti-CD63 antibody [KILL150A] - BSA and Azide free
Description	Mouse monoclonal [KILL150A] to CD63 - BSA and Azide free
Host species	Mouse
Specificity	We recommend **ab134045** for low expression samples.
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt, IHC-P
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 (boiled and un-boiled). Human lymph node tissue lysate. IHC-P: Human melanoma tissue. ICC/IF: SK-MEL-28 cells. Flow Cyt: SK-MEL-28
General notes	ab271296 is the carrier-free version of **ab271286**.

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This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**.

Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

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.

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.

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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	KILL150A
Isotype	IgG

Applications

The Abpromise guarantee   Our **Abpromise guarantee** covers the use of ab271296 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		Use at an assay dependent concentration. Predicted molecular weight: 26 kDa.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

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.
Cellular localization	Cell membrane. Lysosome membrane. Late endosome membrane. Also found in Weibel-Palade bodies of endothelial cells. Located in platelet dense granules.

Images
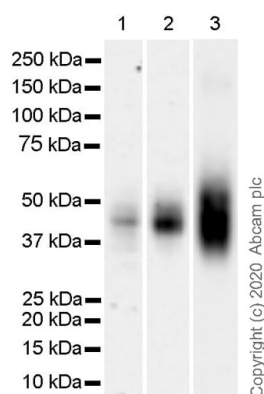
Western blot - Anti-CD63 antibody [KILL150A] - BSA and Azide free (ab271296)

All lanes : Anti-CD63 antibody [KILL150A] (**ab271286**) at 0.978 µg/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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab271286**).
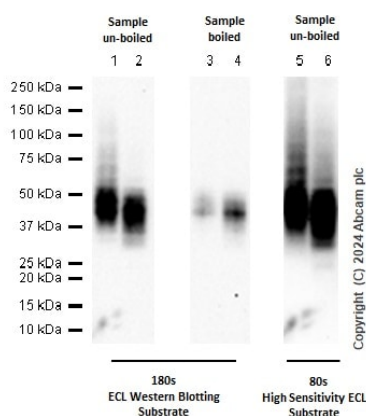
Western blot - Anti-CD63 antibody [KILL150A] - BSA and Azide free (ab271296)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab271286**).
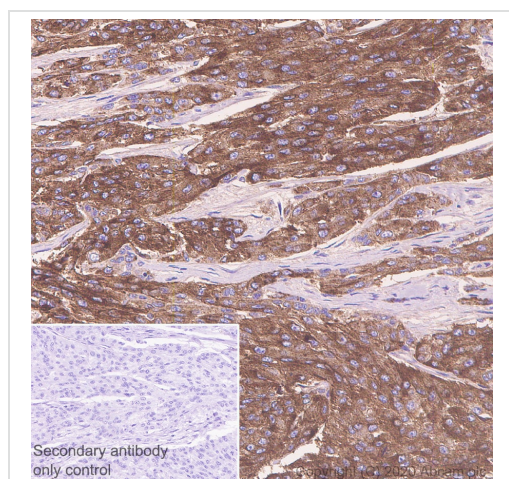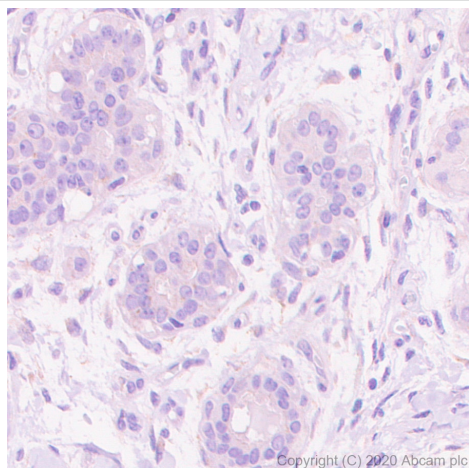

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD63 antibody [KILL150A] - BSA and Azide free (ab271296)

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 IgG 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab271286**).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD63 antibody [KILL150A] - BSA and Azide free (ab271296)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab271286**).
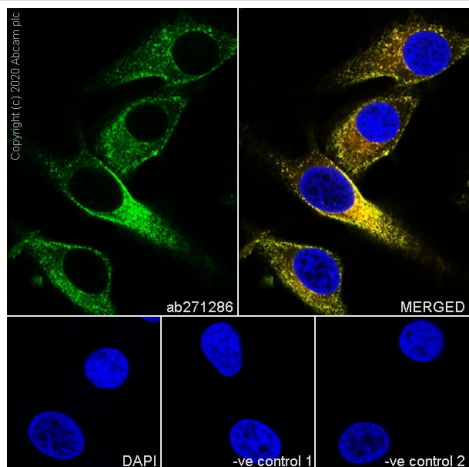
Immunocytochemistry/ Immunofluorescence - Anti-CD63 antibody [KILL150A] - BSA and Azide free (ab271296)
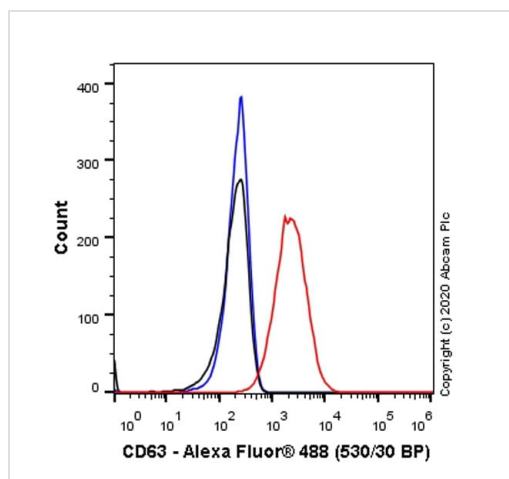
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SK-MEL-28 cells labelling CD63 with **ab271286** at 1/50 dilution (19.56µg/ml), followed by **ab150113** Goat Anti-mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing lysosome/endosomes (**ab252919**) co-staining in SK-MEL-28 cells.

ab252919 Anti-CD63 rabbit monoclonal antibody was used to counterstain tubulin at 1/250 dilution, followed by **ab150080** Goat Anti-Rabbit IgG 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 (19.56µg/ml) followed by **ab150080** at a 1/1000 dilution.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab271286**).

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 IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor$^\circledR$ 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab271286**).

Flow Cytometry - Anti-CD63 antibody [KILL150A] - BSA and Azide free (ab271296)

Anti-CD63 antibody [KILL150A] - BSA and Azide free (ab271296)

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