abcam

Product datasheet

Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free ab213092

Recombinant

7 Images

Overview

Product name	Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free
Description	Mouse monoclonal [MX-49.129.5] to CD63 - BSA and Azide free
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, IHC-P, Flow Cyt, WB, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human
Immunogen	Full length native protein (purified) corresponding to Human CD63 aa 1 to the C-terminus. Database link: **P08962** Run BLAST with Run BLAST with
Positive control	WB: HUVEC and HL-60 cell lysates. IHC-P: Human melanoma tissue. Flow Cyt: SK-MEL-28 and Human peripheral blood mononuclear cells. Flow Cyt (Intra): SK-MEL-28 cells. ICC/IF: SK-MEL-28 cells.
General notes	This product has switched from a hybridoma to recombinant production method on 9th February 2022. 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 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: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	MX-49.129.5
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab213092 in the following tested applications.

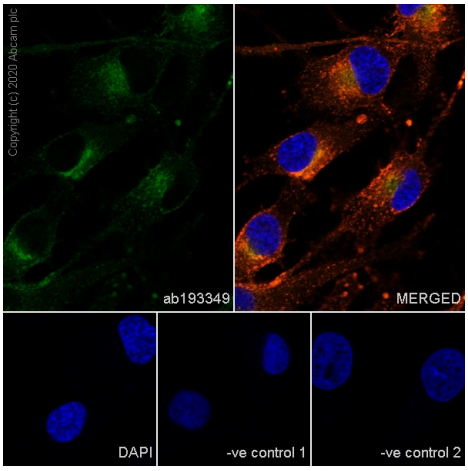
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 26 kDa.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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

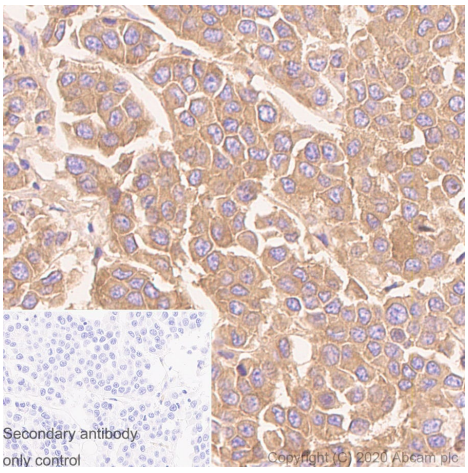


Immunocytochemistry/ Immunofluorescence - Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free (ab213092)

This data was developed using **ab193349**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SK-MEL-28 (Human malignant melanoma) cells labelling CD63 with **ab193349** at 1/50 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (**ab150113**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in SK-MEL-28 cell line. The nuclear counter stain is DAPI (blue).

CD63 is also detected with Rabbit monoclonal Anti-CD63 antibody [EPR22458-280] (**ab252919**) at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (**ab150080**) secondary antibody at 1/500 dilution (red).
The negative controls are as follows:
-ve control 1: **ab193349** at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (**ab150080**) secondary antibody at 1/500 dilution.
-ve control 2: Anti-CD63 antibody [EPR22458-280] (**ab252919**) at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (Alexa Fluor® 488) (**ab150113**) secondary antibody at 1/1000 dilution.

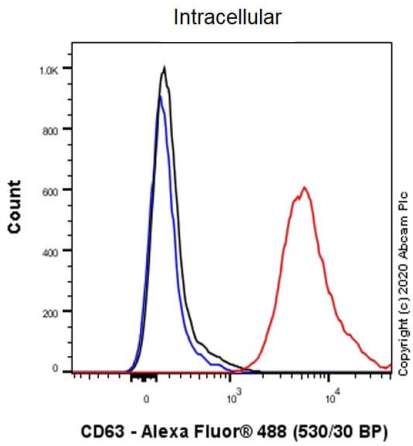


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free (ab213092)

This data was developed using **ab193349**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded human melanoma tissue labelling CD63 with **ab193349** at 1/5000 dilution, followed by Goat Anti-Mouse IgG H&L (HRP polymer) (**ab214879**) at the supplied dilution. Cytoplasmic staining on human melanoma is observed. Counter stained with Hematoxylin. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) was performed for 20 mins, before the section was incubated with **ab193349** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

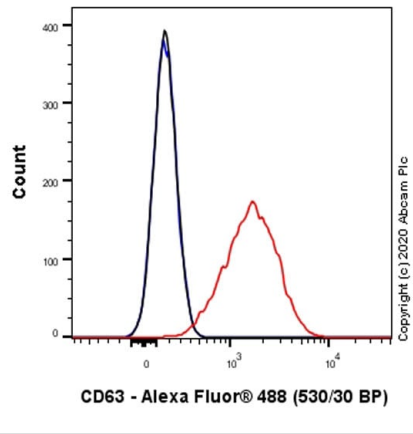
Secondary antibody only control: Used PBS instead of primary antibody.



Flow Cytometry (Intracellular) - Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free (ab213092)

This data was developed using **ab193349**, the same antibody clone in a different buffer formulation.

Flow cytometric (intracellular) analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilised SK-MEL-28 (Human malignant melanoma) cell line labelling CD63 with **ab193349** at 1/1000 dilution (red) compared with a Mouse monoclonal IgG (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Mouse IgG H&L (Alexa Fluor® 488, **ab150113**) at 1/2000 dilution was used as the secondary antibody.

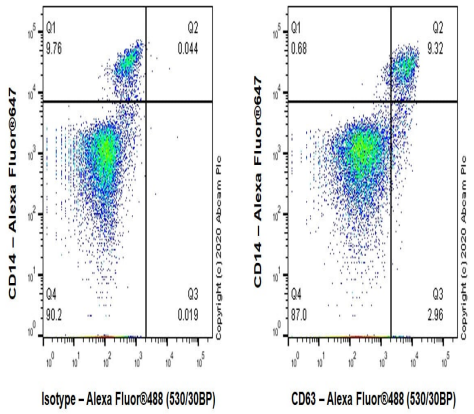


Flow Cytometry - Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free (ab213092)

This data was developed using **ab193349**, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of SK-MEL-28 (Human malignant melanoma) cell line labelling CD63 with **ab193349** at 1/1000 dilution (red) compared with a Mouse monoclonal IgG (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Mouse IgG H&L (Alexa Fluor® 488, **ab150113**) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.

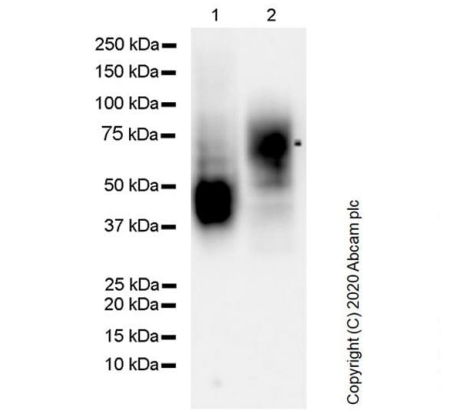


Flow Cytometry - Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free (ab213092)

This data was developed using **ab193349**, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of human peripheral blood mononuclear cells (PBMC) labeling CD63 with **ab193349** at 1/1000 dilution (right), compared with a Mouse monoclonal IgG (left). Goat anti mouse IgG (Alexa Fluor® 488, **ab150113**), at 1/2000 dilution was used as the secondary antibody.

Cells were stained with mouse IgG or **ab193349**, then stained with anti-CD14 conjugated to Alexa Fluor® 647. Gated on viable cells.



Western blot - Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free (ab213092)

All lanes : Anti-CD63 antibody [MX-49.129.5] (**ab193349**) at 1/1000 dilution

Lane 1 : HUVEC (human umbilical vein endothelial cell), whole cell lysate with NFDM/TBST
Lane 2 : HL-60 (human Acute Promyelocytic Leukemia promyeloblast), whole cell lysate with NFDM/TBST

Lysates/proteins at 40 µg per lane.

Blocking peptides at 5 % per lane.

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

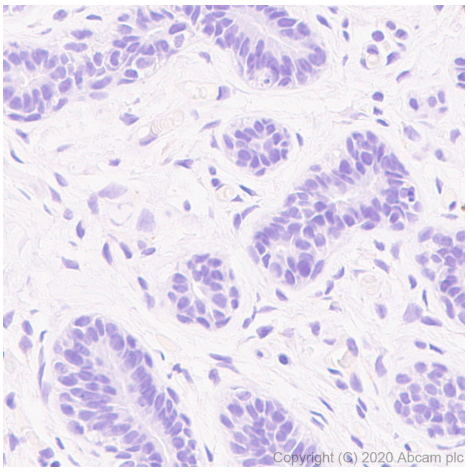
Predicted band size: 26 kDa
Observed band size: 40-60 kDa

Exposure time: 59 seconds

This data was developed using **ab193349**, the same antibody clone in a different buffer formulation.

Diluting buffer and concentration: 5% NFDM/TBST

CD63 can undergo glycosylation as shown in lane 1 and 2 (PMID: 9890706, 28740179).
Samples are non-boiled as boiling may cause protein aggregates.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD63 antibody [MX-49.129.5] - BSA and Azide free (ab213092)

This data was developed using **ab193349**, the same antibody clone in a different buffer formulation.

Negative control staining using **ab193349**. Immunohistochemical analysis of paraffin-embedded human breast tissue labelling CD63 with **ab193349** at 1/5000 dilution, followed by Goat Anti-Mouse IgG H&L (HRP polymer) (**ab214879**) at
the supplied dilution. No staining on human breast is observed, as expected (PMID: 22957045). Counter stained with Hematoxylin. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) was performed for 20 mins, before the section was incubated with **ab193349** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

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