abcam

Product datasheet

Anti-CD9 antibody [EPR23105-121] - BSA and Azide free ab263023

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-CD9 antibody [EPR23105-121] - BSA and Azide free
Description	Rabbit monoclonal [EPR23105-121] to CD9 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, ICC/IF, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A549, MCF7, HCT 116, Human tonsil and Human colon lysates. IHC-P: Human spleen and Human bladder carcinoma tissues. ICC/IF: HCT 116 cells. Flow Cyt:HCT 116 cells. IP: HCT 116 cell lysate.
General notes	ab263023 is the carrier-free version of **ab236630**. 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**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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	EPR23105-121
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab263023 in the following tested applications.

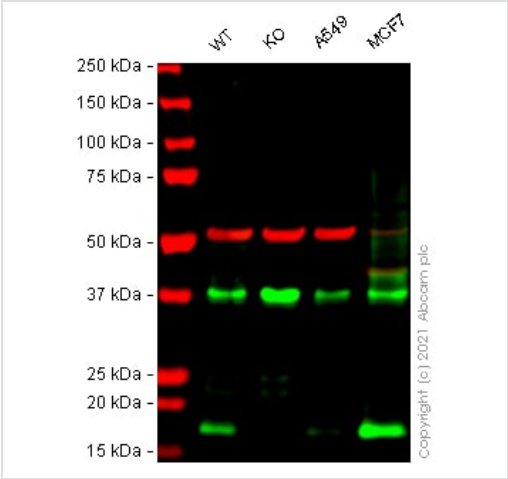
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 22 kDa (predicted molecular weight: 25 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P	★★★★☆ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		Use at an assay dependent concentration.

Target

Function	Involved in platelet activation and aggregation. Regulates paranodal junction formation. Involved in cell adhesion, cell motility and tumor metastasis. Required for sperm-egg fusion.
Tissue specificity	Expressed by a variety of hematopoietic and epithelial cells.
Sequence similarities	Belongs to the tetraspanin (TM4SF) family.
Post-translational modifications	Protein exists in three forms with molecular masses between 22 and 27 kDa, and is known to carry covalently linked fatty acids.
Cellular localization	Membrane.

Images



Western blot - Anti-CD9 antibody [EPR23105-121] - BSA and Azide free (ab263023)

All lanes : Anti-CD9 antibody [EPR23105-121] (**ab236630**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : CD9 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

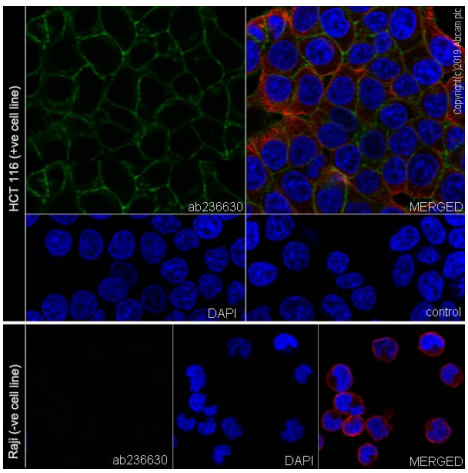
Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 18 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab236630**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab236630** observed at 18 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab236630 was shown to react with CD9 in wild-type HeLa cells in Western blot with loss of signal observed in CD9 knockout cell line **ab255375** (CD9 knockout cell lysate **ab263754**). Wild-type HeLa and CD9 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab236630** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

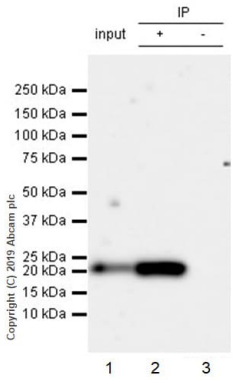


Immunocytochemistry/ Immunofluorescence - Anti-CD9 antibody [EPR23105-121] - BSA and Azide free (ab263023)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (human colorectal carcinoma epithelial cell) cells labelling CD9 with **ab236630** at 1/500 dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in HCT 116 cell line. **Negative control:** Raji (PMID: 8921952). **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab236630**).



Immunoprecipitation - Anti-CD9 antibody [EPR23105-121] - BSA and Azide free (ab263023)

CD9 was immunoprecipitated from 0.35 mg HCT 116 (human colorectal carcinoma epithelial cell) whole cell lysate with **ab236630** at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab236630** at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/1000 dilution.

Lane 1: HCT 116 (human colorectal carcinoma epithelial cell) whole cell lysate 10ug.

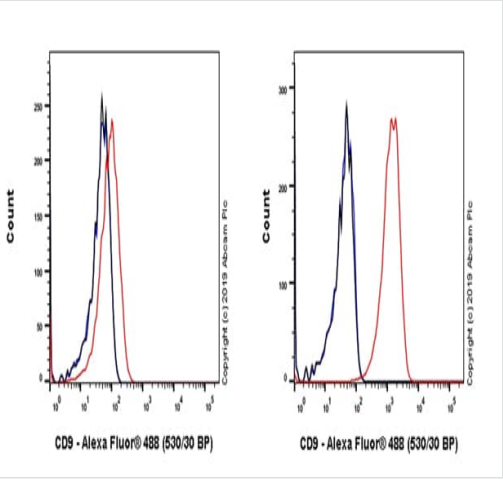
Lane 2: **ab236630** IP in HCT 116 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab236630** in HCT 116 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab236630**).



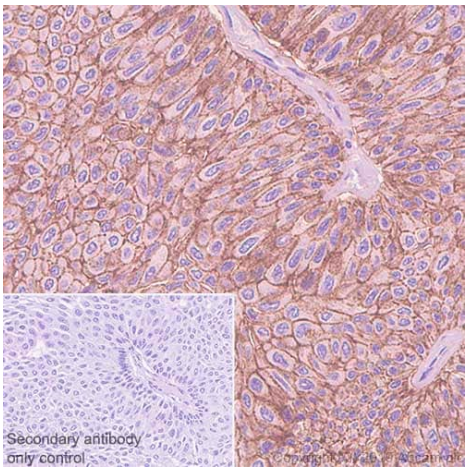
Flow Cytometry - Anti-CD9 antibody [EPR23105-121] - BSA and Azide free (ab263023)

Flow cytometric analysis of Raji (human Burkitt's lymphoma B lymphocyte, Left) / HCT 116 (human colorectal carcinoma epithelial cell, Right) cells labelling CD9 with **ab236630** at 1/50 dilution 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® 488, **ab150077**) at 1/2000 was used as the secondary antibody.

Negative control: Raji (PMID: 8921952).

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 (**ab236630**).



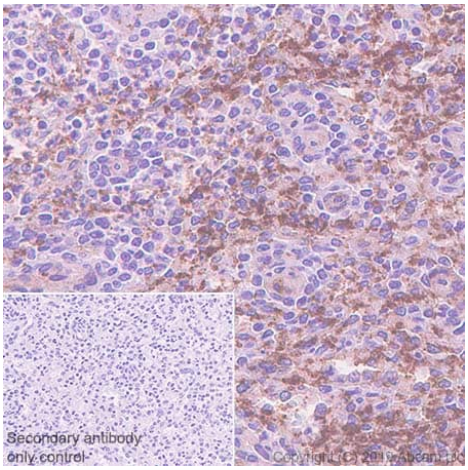
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD9 antibody [EPR23105-121] - BSA and Azide free (ab263023)

Immunohistochemical analysis of paraffin-embedded Human bladder carcinoma tissue labeling CD9 with **ab236630** at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human bladder carcinoma. The section was incubated with **ab236630** 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 a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab236630**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD9 antibody [EPR23105-121] - BSA and Azide free (ab263023)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling CD9 with **ab236630** at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on platelets of human spleen. The section was incubated with **ab236630** 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 a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab236630**).

Anti-CD9 antibody [EPR23105-121] - BSA and Azide free (ab263023)

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