abcam

Product datasheet

Anti-ICAM1 antibody [EPR4776] - BSA and Azide free ab226059

Recombinant RabMAb

9 Images

Overview

Product name	Anti-ICAM1 antibody [EPR4776] - BSA and Azide free
Description	Rabbit monoclonal [EPR4776] to ICAM1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment within Human ICAM1 aa 300-500 (C terminal). The exact sequence is proprietary. Database link: **P05362**
Positive control	IHC-P: Human lung carcinoma and tonsil tissue sections; Flow Cyt (intra): Ramos cells; ICC/IF: Raji cells; IP: Raji whole cell lysate; WB: HUVEC treated with 10ng/ml for 18 hours and Ramos whole cell lysate.
General notes	ab226059 is the carrier-free version of **ab109361**. 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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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	EPR4776
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab226059 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 89 kDa (predicted molecular weight: 58 kDa).
IP		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. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an assay dependent concentration.

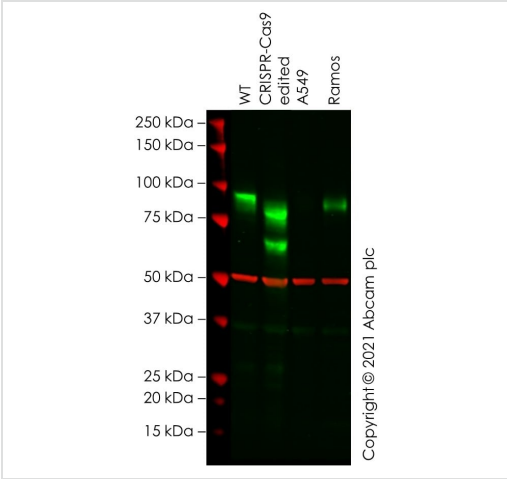
Target

Function ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin alpha-L/beta-2). During leukocyte trans-endothelial migration, ICAM1 engagement promotes the assembly of endothelial apical cups through ARHGEF26/SGEF and RHOG activation. In case of rhinovirus infection acts as a cellular receptor for the virus.

Sequence similarities Belongs to the immunoglobulin superfamily. ICAM family.

	Contains 5 Ig-like C2-type (immunoglobulin-like) domains.
Post-translational modifications	Monoubiquitinated, which is promoted by MARCH9 and leads to endocytosis.
Cellular localization	Membrane.

Images



Western blot - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

All lanes : Anti-ICAM1 antibody [EPR4776] (**ab109361**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ICAM1 CRISPR-Cas9 edited HeLa cell lysate
Lane 3 : A549 cell lysate
Lane 4 : Ramos cell lysate

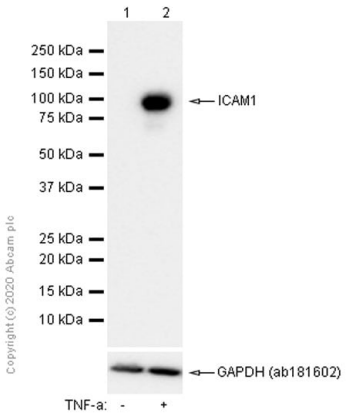
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 90 kDa

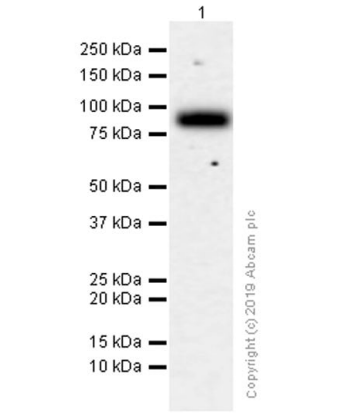
False colour image of Western blot: Anti-ICAM1 antibody [EPR4776] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab109361** was shown to bind specifically to ICAM1. A band was observed at 90 kDa in wild-type HeLa cell lysates with no signal observed at this size in Icam1 knockout cell line **ab261742** (knockout cell lysate **ab256947**). The band observed in the CRISPR-Cas9 edited lysate lane below 90 kDa is likely to represent a truncated form of ICAM1. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and Icam1 CRISPR-Cas9 edited HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L

(IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

This data was developed using **ab109361**, the same antibody clone in a different buffer formulation.



Western blot - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

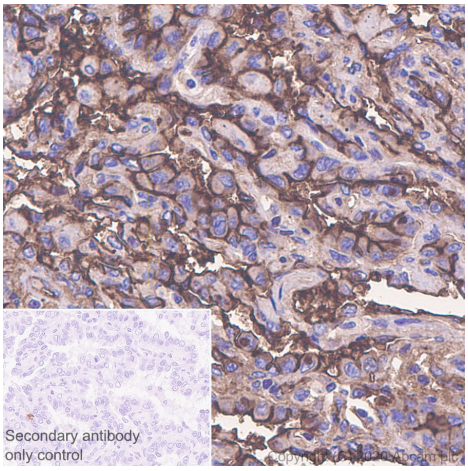
Anti-ICAM1 antibody [EPR4776] (**ab109361**) at 1/1000 dilution (Purified) + Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

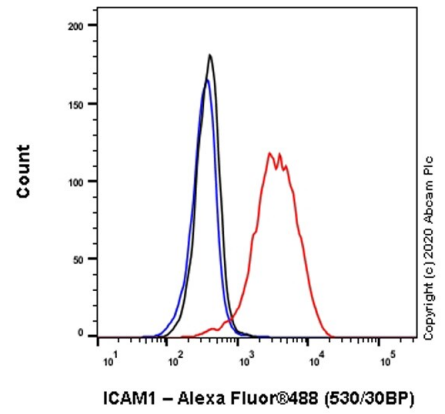
Predicted band size: 58 kDa

This data was developed using **ab109361**, the same antibody clone in a different buffer formulation.



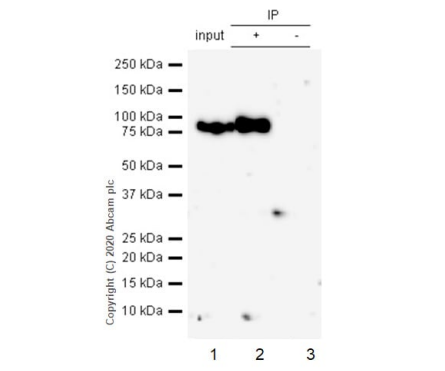
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

This data was developed using **ab109361**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue sections labeling ICAM1 with Purified **ab109361** at 1/100 dilution (6.79 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



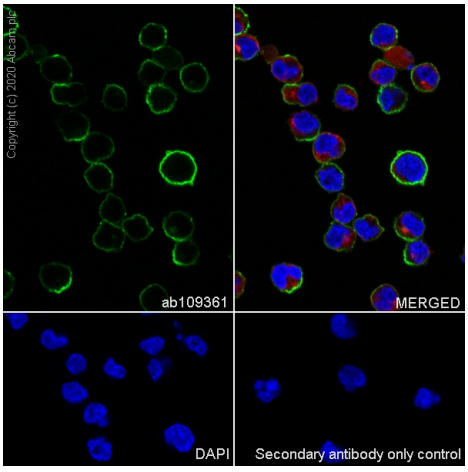
Flow Cytometry (Intracellular) - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

This data was developed using **ab109361**, the same antibody clone in a different buffer formulation. Intracellular Flow Cytometry analysis of Ramos (Human Burkitt's lymphoma B lymphocyte) cells labeling ICAM1 with Purified **ab109361** at 1/70 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



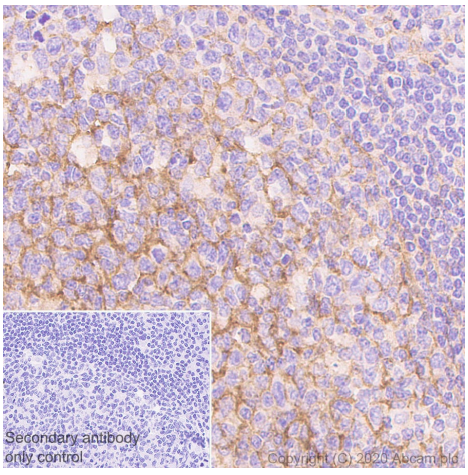
Immunoprecipitation - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

This data was developed using **ab109361**, the same antibody clone in a different buffer formulation.
Purified **ab109361** at 1/30 dilution (2µg) immunoprecipitating ICAM1 in Raji whole cell lysate.
Lane 1 (input): Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate 10µg
Lane 2 (+): **ab109361** + Raji whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab109361** in Raji whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 89 kDa



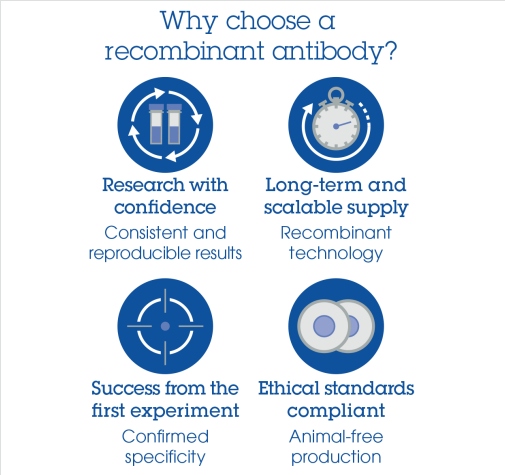
Immunocytochemistry/ Immunofluorescence - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

This data was developed using **ab109361**, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of Raji (Human Burkitt's lymphoma B lymphocyte) cells labeling ICAM1 with Purified **ab109361** at 1/100 dilution (6.79 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 dilution (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 dilution (2 µg/mL). DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

This data was developed using **ab109361**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling ICAM1 with Purified **ab109361** at 1/100 dilution (6.79 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Anti-ICAM1 antibody [EPR4776] - BSA and Azide free (ab226059)

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