abcam

Product datasheet

Anti-ICAM1 antibody [EP1442Y] ab53013

Recombinant RabMAb

56 References 6 Images

Overview

Product name Anti-ICAM1 antibody [EP1442Y]

Description Rabbit monoclonal [EP1442Y] to ICAM1

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Species reactivity Reacts with: Human

Immunogen Synthetic peptide within Human ICAM1 aa 1-100 (N terminal). The exact sequence is proprietary.

(Peptide available as ab218845)

Positive control WB: Huvec, Ramos and Raji whole cell lysate; IHC: Human kidney and tonsil tissue.

General notesThis 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 ${\hbox{\bf RabMAb}^{@}}$ 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 -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EP1442Y

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Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab53013 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 | | 1/1000. Detects a band of approximately 89 kDa (predicted molecular weight: 58 kDa). |
| IHC-P | | 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

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 similaritiesBelongs to the immunoglobulin superfamily. ICAM family.

Contains 5 lg-like C2-type (immunoglobulin-like) domains.

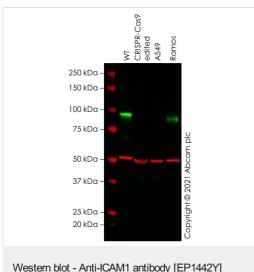
Post-translational

modifications

 $\label{eq:monoubiquitinated} \mbox{Monoubiquitinated, which is promoted by MARCH9 and leads to endocytosis.}$

Cellular localization Membrane.

Images



Western blot - Anti-ICAM1 antibody [EP1442Y] (ab53013)

All lanes: Anti-ICAM1 antibody [EP1442Y] (ab53013) at 1/1000

dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: ICAM1 CRISPR-Cas9 edited HeLa cell lysate

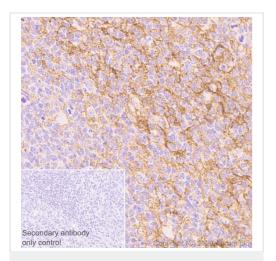
Lane 3 : A549 cell lysate
Lane 4 : Ramos cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

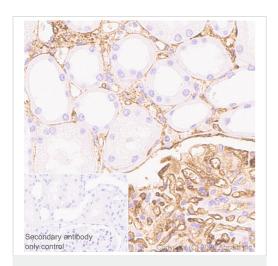
Predicted band size: 58 kDa **Observed band size:** 90 kDa

False colour image of Western blot: Anti-ICAM1 antibody [EP1442Y] 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, ab53013 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 lcam1 CRISPR-Cas9 edited cell line ab261742 (CRISPR-Cas9 edited cell lysate ab256947). The band observed in the CRISPR-Cas9 edited lysate lane below 90 kDa (not observed by this antibody) 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, wildtype and lcam1 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 lgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICAM1 antibody
[EP1442Y] (ab53013)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil tissue sections labeling ICAM1 with purified ab53013 at 1/500 dilution (0.22 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. 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.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICAM1 antibody
[EP1442Y] (ab53013)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human kidney tissue sections labeling ICAM1 with purified ab53013 at 1/500 dilution (0.22 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. 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.



Western blot - Anti-ICAM1 antibody [EP1442Y] (ab53013)

Lanes 1 & 3: Anti-ICAM1 antibody [EP1442Y] (ab53013) at 1/1000 dilution

Lane 2: Anti-ICAM1 antibody [EP1442Y] (ab53013) at 1/1000 dilution (Purified)

Lane 1 : Untreated HUVEC (Human umbilical vein endothelial cell) whole cell lysate

Lane 2 : HUVEC (Human umbilical vein endothelial cell) treated with 50ng/ml TNF-a for 24 hours whole cell lysate

Lane 3 : Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate

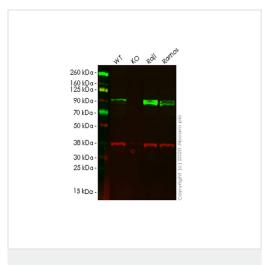
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 58 kDa

The molecular mass observed is consistent with what has been described in the literatures (PMID: 29777158, 30082828, 31244919).



Western blot - Anti-ICAM1 antibody [EP1442Y] (ab53013)

All lanes : Anti-ICAM1 antibody [EP1442Y] (ab53013) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: ICAM1 knockout HeLa cell lysate

Lane 3 : Raji cell lysate

Lane 4 : Ramos cell lysate

Lysates/proteins at 20 µg per lane.

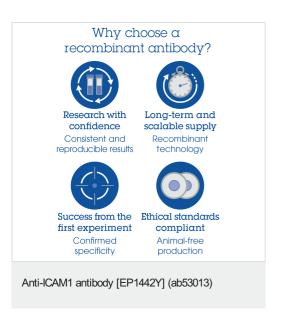
Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

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

Lanes 1-4: Merged signal (red and green). Green - ab53013 observed at 90 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab53013 Anti-ICAM1 antibody [EP1442Y] was shown to specifically react with ICAM1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261742 (knockout cell lysate ab256947) was used. Wild-type and ICAM1 knockout samples were subjected to SDS-PAGE. ab53013 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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