abcam

Product datasheet

Anti-ICAM1 antibody [EPR22161-284] ab222736

Recombinant RabMAb

10 References 7 Images

Overview

Product name Anti-ICAM1 antibody [EPR22161-284]

Description Rabbit monoclonal [EPR22161-284] to ICAM1

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, Flow Cyt, ICC/IF, IP

Species reactivity Reacts with: Mouse

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse kidney, spleen and liver tissue lysate; A20 whole cell lysate. IHC-P: Mouse lung and

spleen tissue. ICC/IF: A20 cells. Flow: Mouse primary splenocytes. IP: Mouse spleen lysate.

General notes 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

Clonality Monoclonal

Clone number EPR22161-284

Isotype ΙgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab222736 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 110 kDa (predicted molecular weight: 57 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.
Flow Cyt		1/500.
ICC/IF		1/50.
IP		1/30.

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.

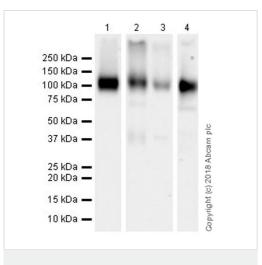
Post-translational

modifications

Monoubiquitinated, which is promoted by MARCH9 and leads to endocytosis.

Cellular localization Membrane.

Images



Western blot - Anti-ICAM1 antibody [EPR22161-284] (ab222736)

All lanes : Anti-ICAM1 antibody [EPR22161-284] (ab222736) at 1/1000 dilution

Lane 1: Mouse kidney tissue lysate

Lane 2: Mouse spleen tissue lysate

Lane 3: Mouse liver tissue lysate

Lane 4: A20 (mouse reticulum sarcoma b lymphocyte) whole cell

lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

1/100000 dilution

Predicted band size: 57 kDa **Observed band size:** 110 kDa

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

Exposure time: 48 seconds.

The 110-KDa apparent molecular mass is due to glycosylation

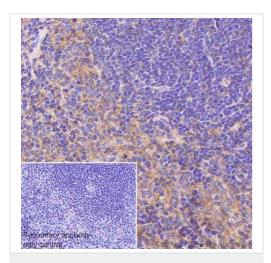
(PMID: 1680698).

Immu
Triton
line) of second showing the tan (PMIC count page).

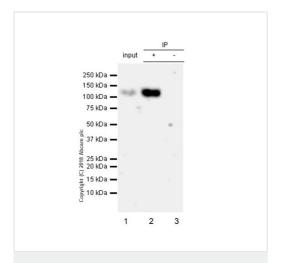
Immunocytochemistry/ Immunofluorescence - Anti-ICAM1 antibody [EPR22161-284] (ab222736)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A20 (mouse reticulum sarcoma cell line) cells labeling ICAM with ab222736 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining in A20 cells with distinct staining of the target protein being transported from Golgi to cell surfaces (PMID: 17486117) is observed. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) was used as a countertsain (Red) at 1/200 dilution. The nuclear counterstain is DAPI (Blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Alexa Fluor[®]488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICAM1 antibody
[EPR22161-284] (ab222736)



Immunoprecipitation - Anti-ICAM1 antibody [EPR22161-284] (ab222736)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling ICAM1 using ab222736 at 1/1000 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on the marginal zone of mouse spleen (PMID: 12130787; PMID: 17947659) is observed. Counterstained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) ready to use.

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

Immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. The section was incubated with ab222736 for 30 mins at room temperature.

ICAM1 was immunoprecipitated from 0.35 mg Mouse spleen lysate with ab222736 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab222736 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1/5000 dilution.

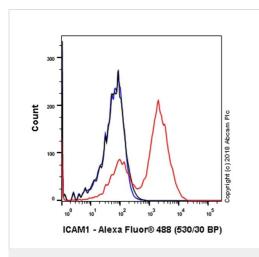
Lane 1: Mouse spleen tissue lysate 10 µg (input).

Lane 2: ab222736 IP in mouse spleen tissue lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab222736 in Mouse spleen lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 3 minutes.

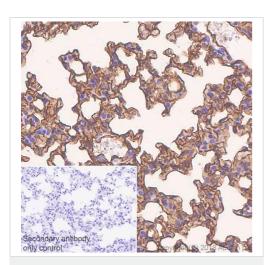
110-kDa molecular mass is due to glycosylation (PMID: 1680698).



Flow Cytometry - Anti-ICAM1 antibody [EPR22161-284] (ab222736)

Flow cytometric analysis of Mouse primary splenocytes labeling ICAM1 with ab222736 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) isotype control (Black) and an unlabeled control (Cell without incubation with primary antibody and secondary antibody, Blue). Goat anti rabbit IgG (Alexa Fluor[®] 488, ab150077) at 1/200 dilution was used as the secondary antibody. Gated on viable cells.

ICAM1 negative population (left half of red peak) was also observed in mouse splenocytes which is consistent with IHC staining pattern.



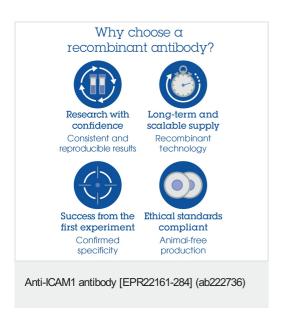
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICAM1 antibody
[EPR22161-284] (ab222736)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling ICAM1 using ab222736 at 1/1000 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Membranous and cytoplasmic staining on mouse lung (PMID: 14568945; PMID: 9802985) is observed. Counterstained with DAPI.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) ready to use.

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

Immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. The section was incubated with ab222736 for 30 mins at room temperature.



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