# abcam

### **Product datasheet**

## Anti-ICAM1 antibody [EPR24639-3] ab282575

КО 評価済 มาวชาวง RabMAb

★★★★★ <u>1 Abreviews</u> <u>4 References</u> 画像数 13

#### 製品の概要

製品名	Anti-ICAM1 antibody [EPR24639-3]
製品の詳細	Rabbit monoclonal [EPR24639-3] to ICAM1
由来種	Rabbit
特異性	Rat species is recommended based on Flow Cyt result. We do not guarantee other applications for rat.
アプリケーション	適用あり: Flow Cyt, IP, IHC-P, ICC/IF, WB 適用なし: IHC-Fr
種交差性	交差種: Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Wild-type HeLa whole cell lysate. Ramos and Raji whole cell lysate. Human kidney tissue lysate. IHC-P: Human kidney tissue. Human breast cancer tissue. ICC/IF: Raji cells. Wild-type HeLa cells. Flow Cyt: Rat splenocytes. C6 and Raji cells. IP: Raji whole cell lysate.
特記事項	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>

製品の特性	
製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
パッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified

ポリ/モノ	モノクローナル
クローン名	EPR24639-3
アイソタイプ	lgG

アプリケーション

The Abpromise guaranteeAbpromise保証は、次のテスト済みアプリケーションにおけるab282575の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

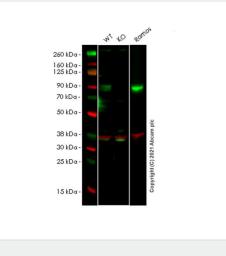
アプリケーション	Abreviews	特記事項
Flow Cyt		1/500.
IP		1/30.
IHC-P		1/600. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
WB	<b>★★★★</b> ★ <u>(1)</u>	1/1000. Predicted molecular weight: 57 kDa.

追加情報

Is unsuitable for IHC-Fr.

ターゲット情報	
機能	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.
配列類似性	Belongs to the immunoglobulin superfamily. ICAM family. Contains 5 lg-like C2-type (immunoglobulin-like) domains.
翻訳後修飾	Monoubiquitinated, which is promoted by MARCH9 and leads to endocytosis.
細胞内局在	Membrane.

画像



Western blot - Anti-ICAM1 antibody [EPR24639-3] (ab282575) All lanes : Anti-ICAM1 antibody [EPR24639-3] (ab282575) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : ICAM1 knockout HeLa whole cell lysate
Lane 3 : Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (IRDye® 800CW) (<u>ab216773</u>) and Goat Anti-Mouse lgG H&L (IRDye® 680RD) (<u>ab216776</u>) at 1/10000 dilution

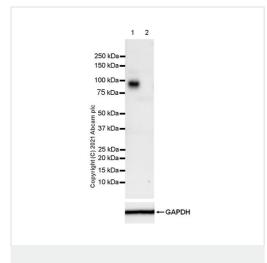
Predicted band size: 57 kDa Observed band size: 90 kDa

Blocking and diluting buffer and concentration: Intercept<sup>®</sup> (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lanes 1-3: Merged signal (red and green). Green - ab282575 observed at 90kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab282575 Anti-ICAM1 antibody [EPR24639-3] was shown to react with ICAM1 in wild-type Hela cells in Western blot. Loss of signal was observed when knockout cell line <u>ab261742</u> (knockout cell lysate <u>ab256947</u>) was used. Wild-type and ICAM1 knockout samples were subjected to SDS-PAGE.

ab282575 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4°C overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preadsorbed (**ab216773**)



Western blot - Anti-ICAM1 antibody [EPR24639-3] (ab282575)

and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-ICAM1 antibody [EPR24639-3] (ab282575) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : ICAM1 knockout Hela whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

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

Predicted band size: 57 kDa Observed band size: 90 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

ab282575 Anti-ICAM1 antibody [EPR24639-3] was shown to react with ICAM1 in wild-type Hela cells in Western blot. Loss of signal was observed when knockout cell line <u>ab261742</u> (knockout cell lysate <u>ab256947</u>) was used. Wild-type and ICAM1 knockout samples were subjected to SDS-PAGE.

Exposure time: 15 seconds.

Anti-ICAM1 antibody [EPR24639-3] (ab282575) at 1/1000 dilution + human kidney tissue lysate at 20 µg

#### Secondary

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution

Predicted band size: 57 kDa Observed band size: 90 kDa

Western blot - Anti-ICAM1 antibody [EPR24639-3] (ab282575)

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250 kDa -

100 kDa

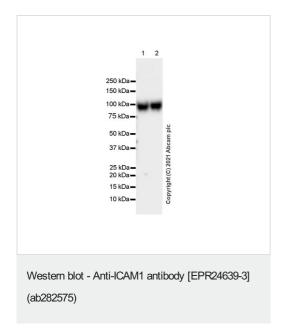
75 kDa

37 kDa – 25 kDa – 20 kDa –

15 kDa **–** 10 kDa **–** 

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 26 seconds



All lanes : Anti-ICAM1 antibody [EPR24639-3] (ab282575) at 1/1000 dilution

Lane 1 : Ramos (human Burkitt's lymphoma B lymphocyte) whole cell lysate

Lane 2 : Raji (human Burkitts lymphoma B lymphocyte) whole cell lysate

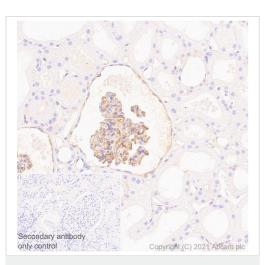
Lysates/proteins at 20 µg per lane.

#### Secondary

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

Predicted band size: 57 kDa Observed band size: 90 kDa

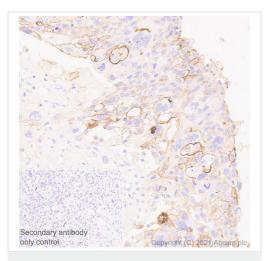
Blocking and diluting buffer and concentration: 5% NFDM/TBST Exposure time: 15 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICAM1 antibody [EPR24639-3] (ab282575) Immunohistochemical analysis of paraffin-embedded human kidney tissue labelling ICAM1 with ab282575 at 1/600 (0.902 µg/ml) dilution followed by a ready to use LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection). Positive staining on normal human glomerulus. The section was incubated with ab282575 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection).

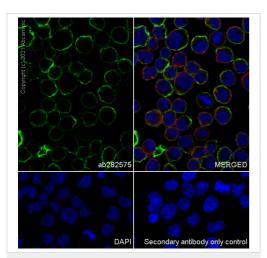
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICAM1 antibody [EPR24639-3] (ab282575)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labelling ICAM1 with ab282575 at 1/600 (0.902 µg/ml) dilution followed by a ready to use LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection). Positive staining on human breast cancer (PMID: 30082828). The section was incubated with ab282575 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

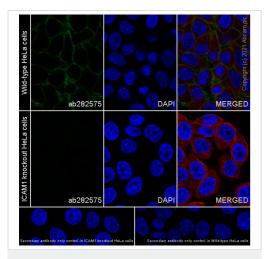


Immunocytochemistry/ Immunofluorescence - Anti-ICAM1 antibody [EPR24639-3] (ab282575)

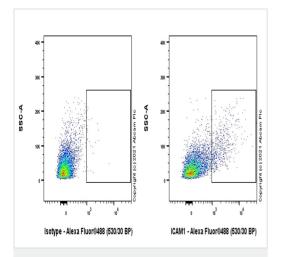
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji cells labelling ICAM1 with ab282575 at 1/500 (1.082 µg/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in Raji cells.

<u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody -Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-ICAM1 antibody [EPR24639-3] (ab282575)



Flow Cytometry - Anti-ICAM1 antibody [EPR24639-3] (ab282575)

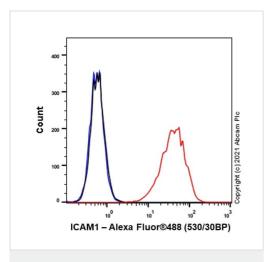
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized ICAM1 KO HeLa cells labelling ICAM1 with ab282575 at 1/500 (1.082 µg/ml) dilution, followed by <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in wild-type HeLa cells, and no staining in ICAM1 knockout HeLa cells is observed.

<u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody -Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution.

Flow cytometric analysis of rat splenocytes cells labelling ICAM1 with ab282575 at 1/500 dilution (0.1  $\mu$ g)/ Right compared with a Rabbit monoclonal IgG (**ab172730**) / Left isotype control. A goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

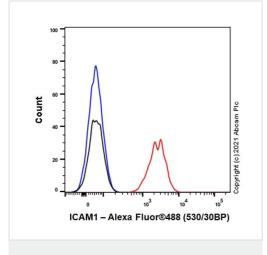
Gated on viable cells.



Flow Cytometry - Anti-ICAM1 antibody [EPR24639-3] (ab282575)

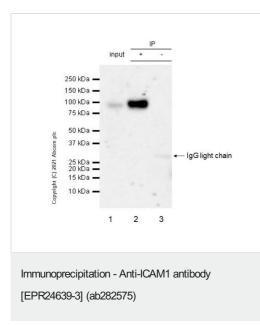
Flow cytometric analysis of C6 (Rat glial tumor glial cell) cells labelling ICAM1 with ab282575 at 1/500 dilution (0.1  $\mu$ g)/(red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.



Flow Cytometry - Anti-ICAM1 antibody [EPR24639-3] (ab282575) Flow cytometric analysis of Raji (Human Burkitt's lymphoma B lymphocyte) cells labelling ICAM1 with ab282575 at 1/500 dilution (0.1 µg)/(Red) compared with a Rabbit monoclonal lgG (<u>ab172730</u>) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.



Why choose a recombinant antibody? Research with Long-term and confidence scalable supply Consistent and Recombinant reproducible results technology Success from the Ethical standards first experiment compliant Confirmed Animal-free specificity production

Anti-ICAM1 antibody [EPR24639-3] (ab282575)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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ICAM1 was immunoprecipitated from 0.35 mg Raji (human burkitt's lymphoma b lymphocyte) whole cell lysate 10 µg with ab282575 at 1/30 dilution (2 µg in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using ab282575 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: Raji whole cell lysate 10 µg

Lane 2: ab282575 IP in Raji whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab282575 in Raji whole cell lysate

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

Exposure time: 3 minutes.

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