

Product datasheet

Anti-CD166 antibody [EPR2759(2)] ab109215

KO VALIDATED Recombinant RabMAB

★★★★☆ 3 Abreviews 16 References 11 Images

Overview

Product name	Anti-CD166 antibody [EPR2759(2)]
Description	Rabbit monoclonal [EPR2759(2)] to CD166
Host species	Rabbit
Tested applications	Suitable for: WB, IP, IHC-P, Flow Cyt, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SH-SY5Y, HuT-78, HT1080, Daudi and HeLa whole cell lysate (ab150035). Mouse and rat brain tissue lysates; Wild-type HAP1 whole cell lysate. IHC-P: Human liver and prostatic adenocarcinoma tissues. ICC/IF: THP-1 cells. Flow Cyt: HuT-78 cells. IP: SH-SY5Y cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p> <p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</p>

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide

	Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2759(2)
Isotype	IgG

Applications

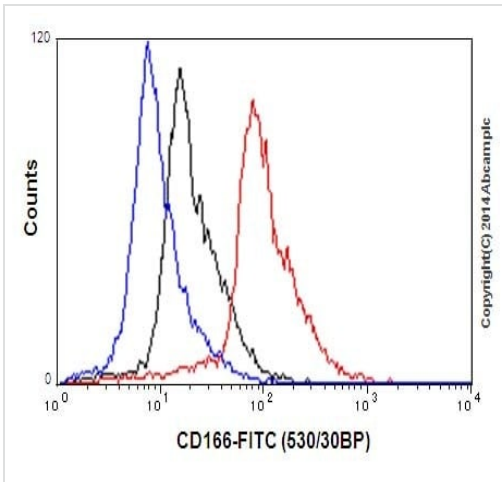
The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab109215 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (1)	1/10000 - 1/20000. Detects a band of approximately 100-105 kDa (predicted molecular weight: 65 kDa). For unpurified use at 1/1000 - 1/10000.
IP	★★★★★ (1)	1/30. For unpurified use at 1/10 - 1/100.
IHC-P	★★★★★ (1)	1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols . For unpurified use at 1/100 - 1/250.
Flow Cyt		1/90. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50 - 1/250.

Target

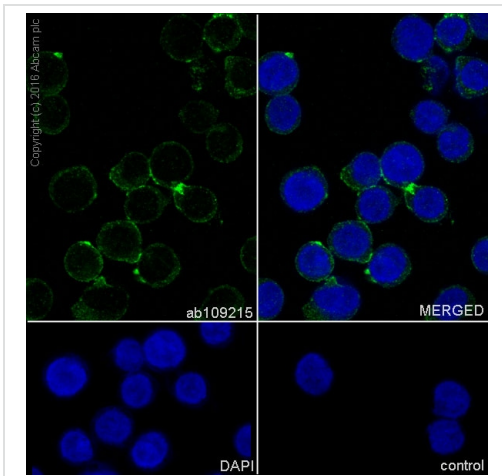
Function	Cell adhesion molecule that binds to CD6. Involved in neurite extension by neurons via heterophilic and homophilic interactions. May play a role in the binding of T- and B-cells to activated leukocytes, as well as in interactions between cells of the nervous system.
Tissue specificity	Spleen, placenta, liver, and weakly in liver. Expressed by activated T-cells, B-cells, monocytes and thymic epithelial cells. Expressed by neurons in the brain. Restricted expression in tumor cell lines. Preferentially expressed in highly metastasizing melanoma cell lines.
Sequence similarities	Contains 3 Ig-like C2-type (immunoglobulin-like) domains. Contains 2 Ig-like V-type (immunoglobulin-like) domains.
Domain	The CD6 binding site is located in the N-terminal Ig-like domain.
Cellular localization	Membrane.

Images



Flow Cytometry - Anti-CD166 antibody [EPR2759(2)] (ab109215)

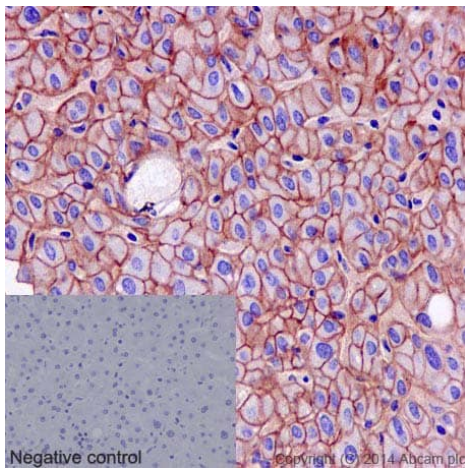
Flow Cytometry analysis of HuT-78 cells labelling CD166 with purified ab109215 at 1/90 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunocytochemistry/ Immunofluorescence - Anti-CD166 antibody [EPR2759(2)] (ab109215)

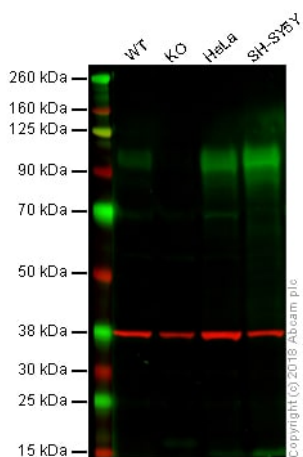
Immunocytochemistry/Immunofluorescence analysis of THP-1 (human monocytic leukemia cell line) cells labelling CD166 (green) with purified ab109215 at 1/250. Cells were fixed with 100% methanol. ab150077, Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as a nuclear counterstain.

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD166 antibody [EPR2759(2)] (ab109215)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling CD166 with purified ab109215 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-CD166 antibody [EPR2759(2)] (ab109215)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

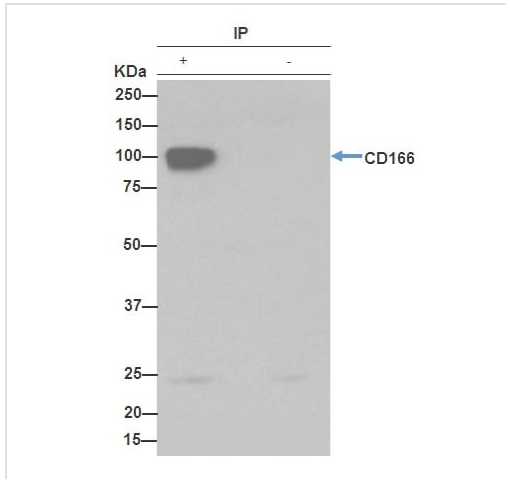
Lane 2: CD166 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: SH-SY5Y whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab109215 observed at 100 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab109215 was shown to specifically react with CD166 in wild-type HAP1 cells as signal was lost in CD166 knockout cells. Wild-type and CD166 knockout samples were subjected to SDS-PAGE. Ab109215 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed ab216772 and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

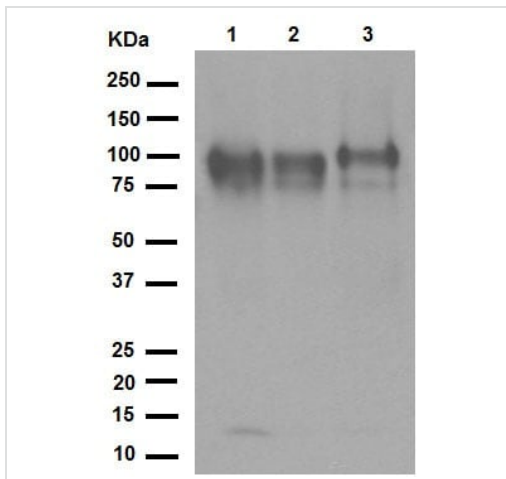


Immunoprecipitation - Anti-CD166 antibody
[EPR2759(2)] (ab109215)

ab109215 (purified) at 1/30 immunoprecipitating CD166 in SH-SY5Y (human neuroblastoma cell line from bone marrow) cell lysate (Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDm/TBST.

Diluting buffer and concentration: 5% NFDm /TBST.



Western blot - Anti-CD166 antibody [EPR2759(2)]
(ab109215)

All lanes : Anti-CD166 antibody [EPR2759(2)] (ab109215) at 1/10000 dilution (purified)

Lane 1 : SH-SY5Y (human neuroblastoma cell line from bone marrow) cell lysate

Lane 2 : HuT-78 cell lysate

Lane 3 : HT-1080 (human fibrosarcoma cell line) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

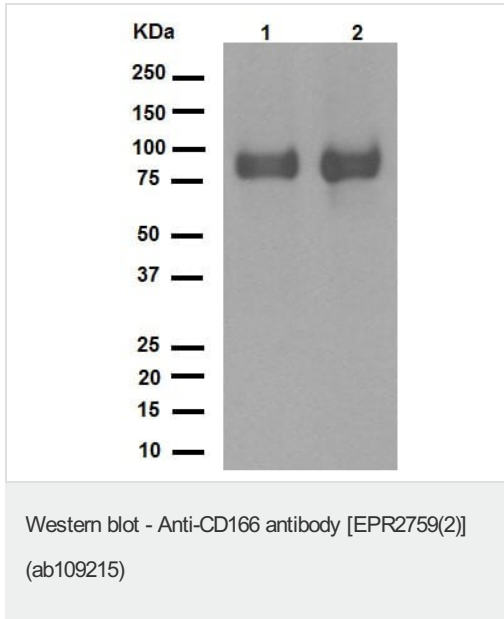
All lanes : Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 65 kDa

Observed band size: 100-105 kDa

Blocking buffer and concentration: 5% NFDm/TBST.

Diluting buffer and concentration: 5% NFDm /TBST.



All lanes : Anti-CD166 antibody [EPR2759(2)] (ab109215) at 1/10000 dilution (purified)

Lane 1 : Mouse brain tissue lysate

Lane 2 : Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

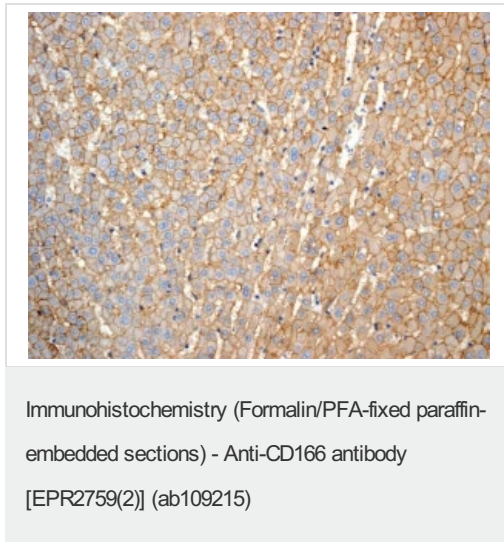
All lanes : Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 65 kDa

Observed band size: 100-105 kDa

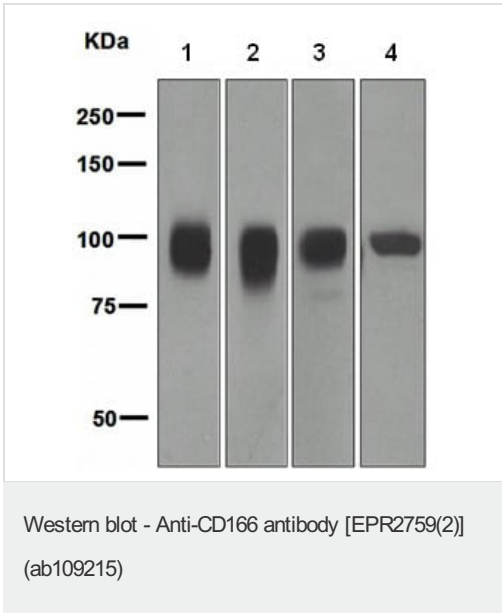
Blocking buffer and concentration: 5% NFDm/TBST.

Diluting buffer and concentration: 5% NFDm /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling CD166 with unpurified ab109215 at 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



All lanes : Anti-CD166 antibody [EPR2759(2)] (ab109215) at 1/1000 dilution (unpurified)

Lane 1 : SH-SY5Y (human neuroblastoma cell line from bone marrow) cell lysate

Lane 2 : HuT-78 cell lysate

Lane 3 : HT1080 (human fibrosarcoma cell line) cell lysate

Lane 4 : Daudi (human Burkitt's lymphoma cell line) cell lysate

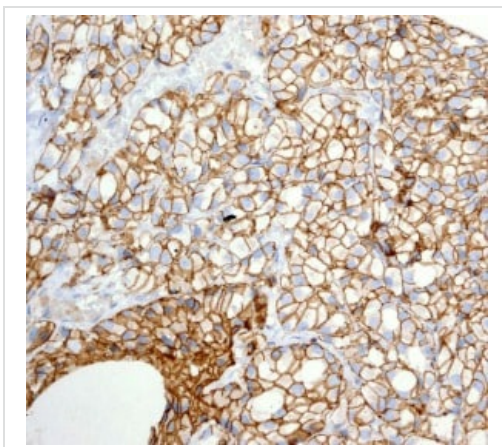
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 65 kDa

Observed band size: 100-105 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostatic adenocarcinoma tissue labelling CD166 with unpurified ab109215 at 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD166 antibody [EPR2759(2)] (ab109215)

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Anti-CD166 antibody [EPR2759(2)] (ab109215)

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