Anti-CD166 Antibody [5-5]

EM1902-36

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 65 kDa.
Clone number:	5-5

Description: Cell adhesion molecule that mediates both heterotypic cell-cell contacts via its interaction with CD6, as well as homotypic cell-cell contacts . Promotes T-cell activation and proliferation via its interactions with CD6 . Contributes to the formation and maturation of the immunological synapse via its interactions with CD6 . Mediates homotypic interactions with cells that express ALCAM . Required for normal hematopoietic stem cell engraftment in the bone marrow . Mediates attachment of dendritic cells onto endothelial cells via homotypic interaction . Inhibits endothelial cell migration and promotes endothelial tube formation via homotypic interactions . Required for normal organization of the lymph vessel network. Required for normal hematopoietic stem cell engraftment in the bone marrow. Plays a role in hematopoiesis; required for normal numbers of hematopoietic stem cells in bone marrow. Promotes in vitro osteoblast proliferation and differentiation (By similarity). Promotes neurite extension, axon growth and axon guidance; axons grow preferentially on surfaces that contain ALCAM. Mediates outgrowth and pathfinding for retinal ganglion cell axons (By similarity).

Immunogen: Recombinant protein within human CD166 aa 1-300.

Positive control: PC-3M cell lysate, A549 cell lysate, SHSY5Y cell lysate, A431cells, Huvec celles, human liver tissue, THP-1 cells.

Subcellular location: Cell membrane, Cell projection, Membrane, Secreted

Database links: SwissProt: Q13740 Human

Recommended Dilutions:

WB	1:500-1:1,000
IHC-P	1:50-1:200
IF-Cell	1:50-1:100
FC	1:50-1:100

Storage Buffer: 1*TBS (pH7.4), 1% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

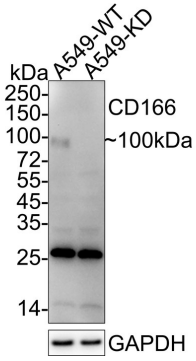

Fig1: Western blot analysis of CD166 on different lysates with Mouse anti-CD166 antibody (EM1902-36) at 1/1,000 dilution.

Lane 1: A549-WT cell lysate
Lane 2: A549-KD CD166 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 65 kDa
Observed band size: 100 kDa

Exposure time: 2 minutes 10 seconds; ECL: K1802;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM1902-36) at 1/1,000 dilution was used in 5% BSA at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

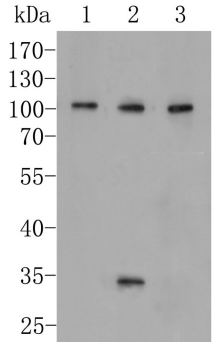

Fig2: Western blot analysis of CD166 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (EM1902-36, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: PC-3M cell lysate
Lane 2: A549 cell lysate
Lane 3: SHSY5Y cell lysate

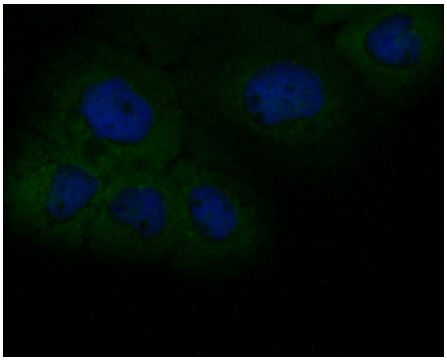

Fig3: ICC staining of CD166 in A431 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (EM1902-36, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Mouse IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

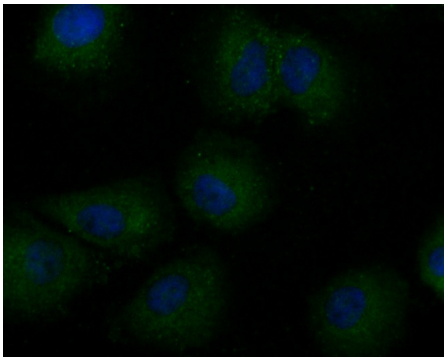

Fig4: ICC staining of CD166 in Huvec cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (EM1902-36, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Mouse IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

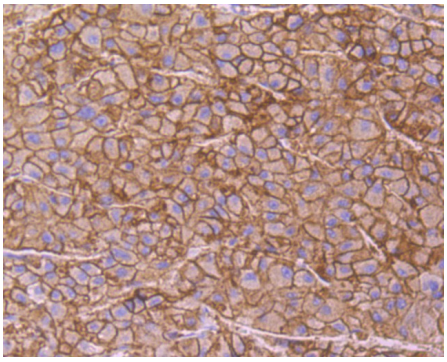

Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue using anti-CD166 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1902-36, 1/100) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

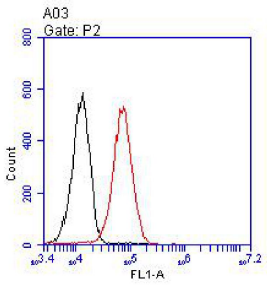

Fig6: Flow cytometric analysis of CD166 was done on THP-1 cells. The cells were fixed, permeabilized and stained with the primary antibody (EM1902-36, 1/100) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated goat anti-Mouse IgG Secondary antibody at 1/500 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Ikeda K.et.al."Molecular isolation and characterization of a soluble isoform of activated leukocyte cell adhesion molecule that modulates endothelial cell function.J. Biol. Chem. 279:55315-55323(2004).