Anti-CD9 Antibody [SA35-08]

ET1601-9

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	SA35-08

Description: CD9 is a gene encoding a protein that is a member of the transmembrane 4 superfamily also known as the tetraspanin family. It is a cell surface glycoprotein that consists of four transmembrane regions and has two extracellular loops that contain disulfide bonds which are conserved throughout the tetraspanin family. Also containing distinct palmitoylation sites that allows CD9 to interact with lipids and other proteins. Tetraspanin proteins are involved in a multitude of biological processes such as adhesion, motility, membrane fusion, signaling and protein trafficking. CD9 has a diverse role in cellular processes as it has also been shown to trigger platelet activation and aggregation. CD9 can also modulate cell adhesion and migration. Additionally, CD9 has been shown to block adhesion of Staphylococcus aureus to wounds. The adhesion is essential for infection of the wound. This suggests that CD9 could be of possible use to as treatment for skin infection by Staphylococcus aureus.

Immunogen: Synthetic peptide within Human CD9 aa 179-228 / 228.

Positive control: HeLa cell lysate, K-562 cell lysate, MCF7 cell lysate, HCT 116 cell lysate, HepG2 cell lysate, SK-MEL-28 cell lysate, A375 cell lysate, B16-F1 cell lysate, SW480, CRC, human tonsil tissue, human spleen tissue, human kidney tissue, mouse brain tissue, mouse spleen tissue.

Subcellular location: Cell membrane, Membrane

Database links: SwissProt: P21926 Human | P40240 Mouse | P40241 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50
IHC-P	1:200-1:1,000
IP	1-2μg/sample
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

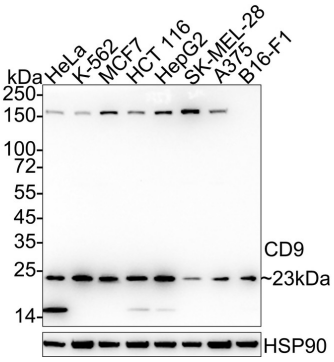

Fig1: Western blot analysis of CD9 on different lysates with Rabbit anti-CD9 antibody (ET1601-9) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: K-562 cell lysate
Lane 3: MCF7 cell lysate
Lane 4: HCT 116 cell lysate
Lane 5: HepG2 cell lysate
Lane 6: SK-MEL-28 cell lysate
Lane 7: A375 cell lysate
Lane 8: B16-F1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 25 kDa
Observed band size: 23 kDa

Exposure time: 3 minutes 30 seconds;
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 (ET1601-9) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

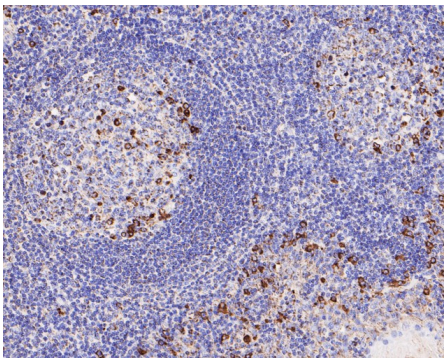

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-CD9 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-9, 1/200) 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.

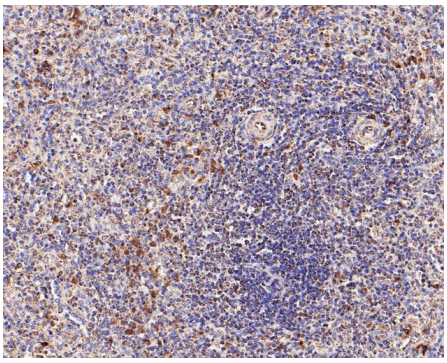

Fig3: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-CD9 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-9, 1/200) 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.

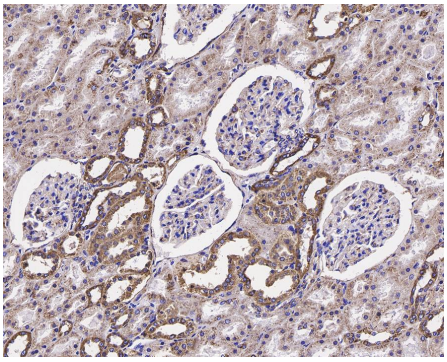

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-CD9 antibody (ET1601-9) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-9) at 1/1,000 dilution for 1 hour 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.

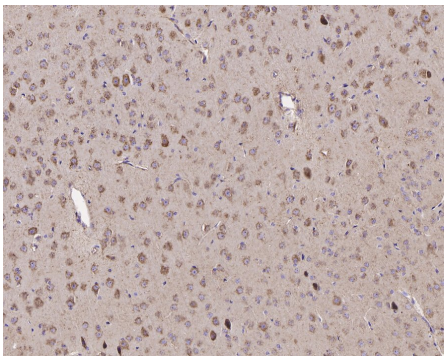

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-CD9 antibody (ET1601-9) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-9) at 1/1,000 dilution for 1 hour 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.

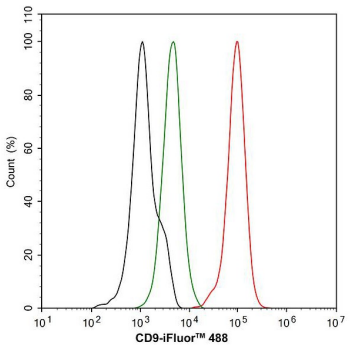

Fig6: Flow cytometric analysis of B16-F1 cells labeling CD9.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (ET1601-9, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

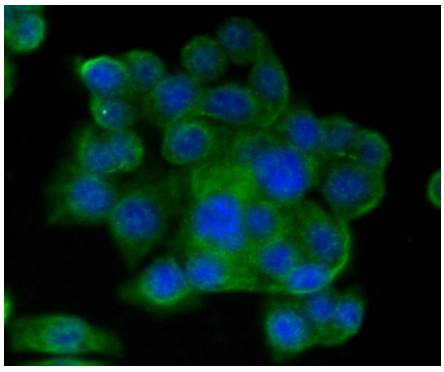

Fig7: ICC staining of CD9 in SW480 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1601-9, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

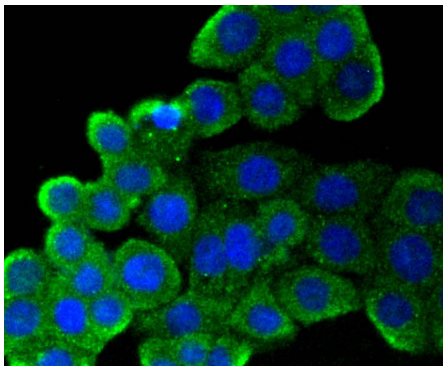

Fig8: ICC staining of CD9 in CRC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1601-9, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

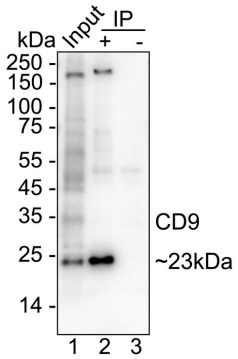

Fig9: CD9 was immunoprecipitated from 0.2 mg K-562 cell lysate with ET1601-9 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1601-9 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: K-562 cell lysate (input)
Lane 2: ET1601-9 IP in K-562 cell lysate
Lane 3: Rabbit IgG instead of ET1601-9 in K-562 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 11 seconds; ECL: K1801

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

Background References

1. Haug, B.H. et al. 2015. Exosome-like Extracellular Vesicles from MYCN-amplified Neuroblastoma Cells Contain Oncogenic miRNAs. Anticancer research. 35: 2521-30.
2. Gallart-Palau, X. et al. 2015. Extracellular vesicles are rapidly purified from human plasma by PRotein Organic Solvent PRecipitation (PROSPR). Scientific reports. 5: 14664.