Anti-NCAM1 / CD56 Antibody [PSH06-82] - BSA and Azide free

HA751104

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IHC-P, IHC-Fr, IF-Cell, FC, IF-Tissue
Molecular Wt:	Predicted band size: 95 kDa
Clone number:	PSH06-82

Description: Neural cell adhesion molecule (NCAM), also called CD56, is a homophilic binding glycoprotein expressed on the surface of neurons, glia and skeletal muscle. Although CD56 is often considered a marker of neural lineage commitment due to its discovery site, CD56 expression is also found in, among others, the hematopoietic system. Here, the expression of CD56 is mostly associated with, but not limited to, natural killer cells. CD56 has been detected on other lymphoid cells, including gamma delta (γδ) T cells and activated CD8+ T cells, as well as on dendritic cells. NCAM has been implicated as having a role in cell–cell adhesion, neurite outgrowth, synaptic plasticity, and learning and memory.

Immunogen: Recombinant protein within human NCAM1 aa 1-739.

Positive control: SH-SY5Y cell lysate, Neuro-2a cell lysate, C6 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, mouse brain tissue, rat brain tissue, SH-SY5Y, Neuro-2a, C6, human peripheral blood lymphocytes.

Subcellular location: Cell membrane.

Database links: SwissProt: P13591 Human | P13595 Mouse | P13596 Rat

Recommended Dilutions:

WB	1:3,000
IHC-P	1:1,000
IHC-Fr	1:500
IF-Cell	1:100-1:1,000
FC	1:1,000
IF-Tissue	1:100

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

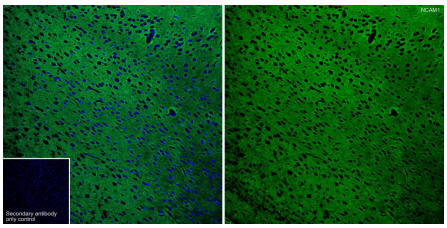

Fig1: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1:500

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

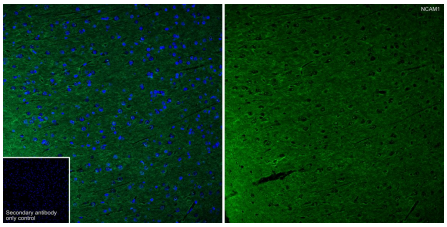

Fig2: Application: IHC-Fr

Species: Rat

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1:500

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

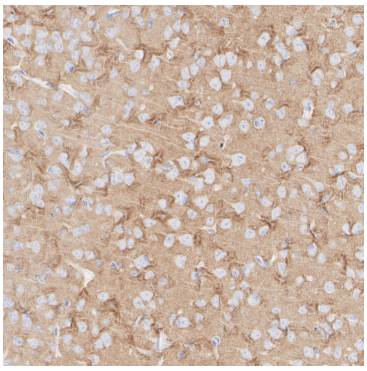

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/1,000 dilution.

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 (HA751104) 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.

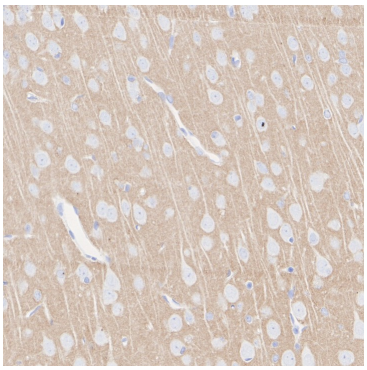

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/1,000 dilution.

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 (HA751104) 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.

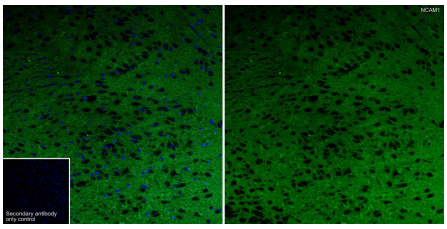

Fig5: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling NCAM1 / CD56 with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA751104, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

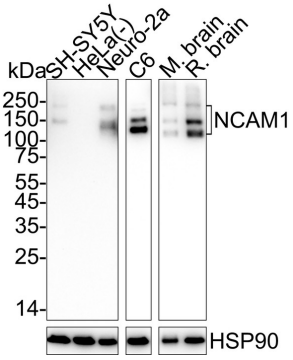

Fig6: Western blot analysis of NCAM1 / CD56 on different lysates with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/3,000 dilution.

Lane 1: SH-SY5Y cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (negative) (20 µg/Lane)
Lane 3: Neuro-2a cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)
Lane 5: Mouse brain tissue lysate (20 µg/Lane)
Lane 6: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 95 kDa
Observed band size: 120-250 kDa

Exposure time: Lane 1-3: 3 minutes; Lane 4-6: 30 seconds; ECL: K1801;
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 (HA751104) at 1/3,000 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.

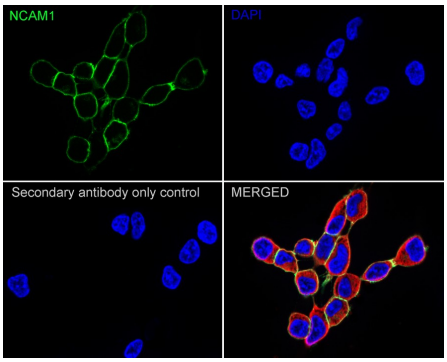

Fig7: Immunocytochemistry analysis of SH-SY5Y cells labeling NCAM1 / CD56 with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/1,000 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

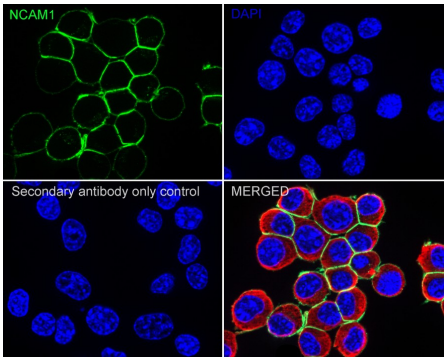

Fig8: Immunocytochemistry analysis of Neuro-2a cells labeling NCAM1 / CD56 with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

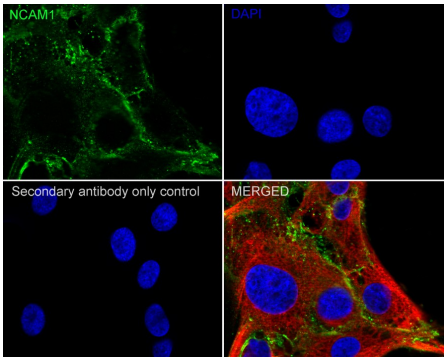

Fig9: Immunocytochemistry analysis of C6 cells labeling NCAM1 / CD56 with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NCAM1 / CD56 antibody (HA751104) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

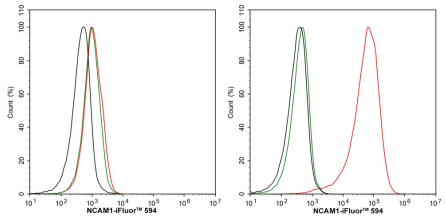

Fig10: Flow cytometric analysis of HeLa (left, negative) and SH-SY5Y (right, positive) cells labeling NCAM1 / CD56.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA751104, 1/1,000) (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™ 594 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1122) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

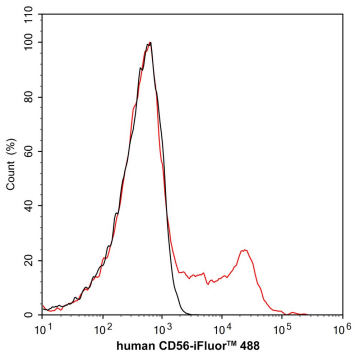

Fig11: Flow cytometric analysis of human peripheral blood lymphocytes labeling NCAM1 / CD56.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA751104, 1μg/mL) (red). 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).

Background References

1. Shiwaku H et al. Autoantibodies against NCAM1 from patients with schizophrenia cause schizophrenia-related behavior and changes in synapses in mice. Cell Rep Med. 2022 Apr
2. Jennings MJ et al. NCAM1 and GDF15 are biomarkers of Charcot-Marie-Tooth disease in patients and mice. Brain. 2022 Nov