Anti-CD133 Antibody [A8C3]

HA601024

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P
Molecular Wt:	Predicted band size: 97 kDa
Clone number:	A8C3

Description: CD133 antigen, also known as prominin-1, is a glycoprotein that in humans is encoded by the PROM1 gene. It is a member of pentaspan transmembrane glycoproteins, which specifically localize to cellular protrusions. When embedded in the cell membrane, the membrane topology of prominin-1 is such that the N-terminus extends into the extracellular space and the C-terminus resides in the intracellular compartment. The protein consists of five transmembrane segments, with the first and second segments and the third and fourth segments connected by intracellular loops while the second and third as well as fourth and fifth transmembrane segments are connected by extracellular loops. CD133 is the most commonly used marker for isolation of cancer stem cell (CSC) population from different tumors, mainly from various gliomas and carcinomas. CD133+ melanoma cells are considered a subpopulation of CSC and play a critical role in recurrence. Moreover, CD133+ melanoma cells are immunogenic and can be used as an antimelanoma vaccination. In mice the vaccination with CD133+ melanoma cells mediated strong anti-tumor activity that resulted in the eradication of parental melanoma cells. In addition, it has also been shown that CD133+ melanoma cells preferentially express the RNA helicase DDX3X . As DDX3X also is an immunogenic protein, the same anti-melanoma vaccination strategy can be employed to give therapeutic antitumor immunity in mice.

Immunogen:	Recombinant protein within human CD133 aa 151-400/865.
Positive control:	Caco-2 cell lysate, HT-29 cell lysate, NCCIT cell lysate, human kidney tissue lysates, human colon carcinoma tissue, human breast tissue, human kidney tissue.
Subcellular location:	Endoplasmic reticulum. Plasma membrane. Cell projection.
Database links:	SwissProt: O43490 Human
Recommended Dilutions:	
WB	1:2,000
IHC-P	1:600
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% 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 G affinity purified.

Images

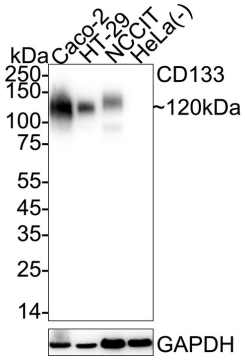

Fig1: Western blot analysis of CD133 on different lysates with Mouse anti-CD133 antibody (HA601024) at 1/2,000 dilution.

Lane 1: Caco-2 cell lysate
Lane 2: HT-29 cell lysate
Lane 3: NCCIT cell lysate
Lane 4: HeLa cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 97 kDa
Observed band size: 120 kDa

Exposure time: 6 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 (HA601024) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

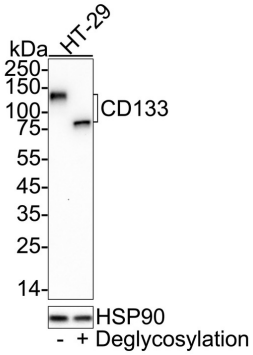

Fig2: Western blot analysis of CD133 on different lysates with Mouse anti-CD133 antibody (HA601024) at 1/2,000 dilution.

Lane 1: HT-29 cell lysate
Lane 2: HT-29 cell lysate treated with deglycosylation

Lysates/proteins at 20 µg/Lane.

Predicted band size: 97 kDa
Observed band size: 120/97 kDa

Exposure time: 1 minute; 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 (HA601024) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

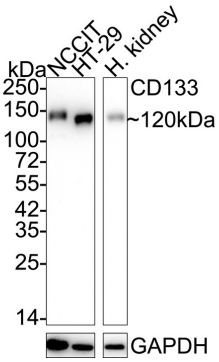

Fig3: Western blot analysis of CD133 on different lysates with Mouse anti-CD133 antibody (HA601024) at 1/1,000 dilution.

Lane 1: NCCIT cell lysate (10 µg/Lane)
Lane 2: HT-29 cell lysate (10 µg/Lane)
Lane 3: Human kidney tissue lysate (20 µg/Lane)

Predicted band size: 97 kDa
Observed band size: 120 kDa

Exposure time: 3 minutes; 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 (HA601024) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

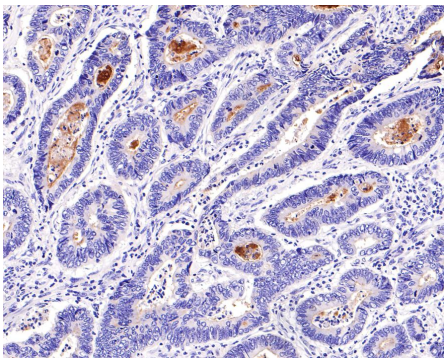

Fig4: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Mouse anti-CD133 antibody (HA601024) at 1/600 dilution.

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 (HA601024) at 1/600 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.

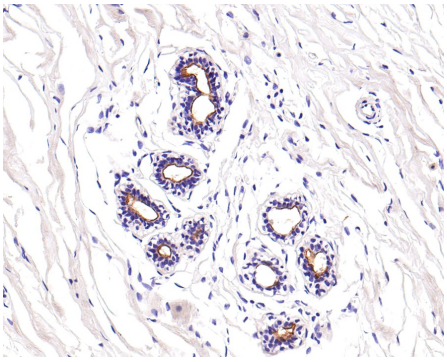

Fig5: Immunohistochemical analysis of paraffin-embedded human breast tissue with Mouse anti-CD133 antibody (HA601024) at 1/600 dilution.

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 (HA601024) at 1/600 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.

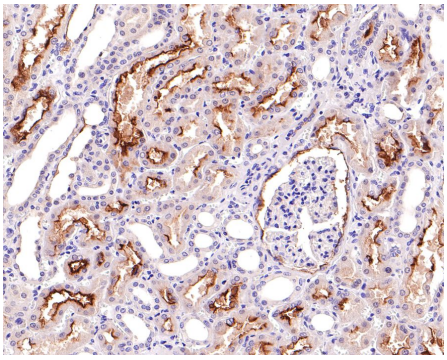

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-CD133 antibody (HA601024) at 1/600 dilution.

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 (HA601024) at 1/600 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.

Background References

1. Kim MY et al. Accumulation of low-dose BIX01294 promotes metastatic potential of U251 glioblastoma cells. Oncol Lett 13:1767-1774 (2017).
2. Xi G et al. Targeting CD133 improves chemotherapeutic efficacy of recurrent pediatric pilocytic astrocytoma following prolonged chemotherapy. Mol Cancer 16:21 (2017).