abcam

Product datasheet

Anti-CD133 antibody - Stem Cell Marker ab19898

Overview

Product name Anti-CD133 antibody - Stem Cell Marker

Description Rabbit polyclonal to CD133 - Stem Cell Marker

Host species Rabbit

Specificity From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and

expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help. You may also be interested in our

alternative recombinant antibody, ab284389.

Tested applications Suitable for: ICC, ICC/IF, Flow Cyt, IHC-P, IHC-Fr, IP, WB

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide corresponding to Human CD133 (C terminal) conjugated to keyhole limpet

haemocyanin.

Database link: **O43490**

Positive control WB: Caco 2, HeLa and human embryonic stem cell lysates. ICC/IF: Caco-2 cells

General notesThe Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Storage instructions Store at +4°C short term (1-2 weeks). Aliquot and store at -20°C or -80°C. Avoid repeated freeze

/ thaw cycles.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituents: PBS, 1% BSA

Purity Immunogen affinity purified

1

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab19898 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
ICC	★★★★ (1)	Use at an assay dependent concentration.
ICC/IF	★★★★★ (6)	Use at an assay dependent concentration.
Flow Cyt	★★★★★ (3)	Use at an assay dependent concentration.
IHC-P	★★★★ <u>(11)</u>	Use at an assay dependent concentration.
IHC-Fr	★★★★ (4)	Use at an assay dependent concentration. PubMed: 23769181
IP		1/30.
WB	*** <u>*</u> (2)	Use a concentration of 1 - 2 μg/ml. Detects a band of approximately 110 kDa (predicted molecular weight: 97 kDa).

Target

Function

May play a role in cell differentiation, proliferation and apoptosis (PubMed:24556617). Binds cholesterol in cholesterol-containing plasma membrane microdomains and may play a role in the organization of the apical plasma membrane in epithelial cells. During early retinal development acts as a key regulator of disk morphogenesis. Involved in regulation of MAPK and Akt signaling pathways. In neuroblastoma cells suppresses cell differentiation such as neurite outgrowth in a RET-dependent manner (PubMed:20818439).

Tissue specificity

Isoform 1 is selectively expressed on CD34 hematopoietic stem and progenitor cells in adult and fetal bone marrow, fetal liver, cord blood and adult peripheral blood. Isoform 1 is not detected on other blood cells. Isoform 1 is also expressed in a number of non-lymphoid tissues including retina, pancreas, placenta, kidney, liver, lung, brain and heart. Found in saliva within small membrane particles. Isoform 2 is predominantly expressed in fetal liver, skeletal muscle, kidney, and heart as well as adult pancreas, kidney, liver, lung, and placenta. Isoform 2 is highly expressed in fetal liver, low in bone marrow, and barely detectable in peripheral blood. Isoform 2 is expressed on hematopoietic stem cells and in epidermal basal cells (at protein level). Expressed in adult retina by rod and cone photoreceptor cells (at protein level).

Involvement in disease

Retinitis pigmentosa 41 Cone-rod dystrophy 12 Stargardt disease 4 Retinal macular dystrophy 2

Sequence similarities

Belongs to the prominin family.

Post-translational

Isoform 1 and isoform 2 are glycosylated.

modifications

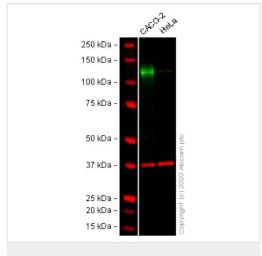
Cellular localization

Acetylation at Lys-225, Lys-257 and Lys-264 by NAT8 and NAT8B may control PROM1 protein

expression and its function in cell apoptosis.

Apical cell membrane. Cell projection, microvillus membrane. Cell projection, cilium, photoreceptor outer segment. Endoplasmic reticulum. Endoplasmic reticulum-Golgi intermediate compartment. Found in extracellular membrane particles in various body fluids such as cerebrospinal fluid, saliva, seminal fluid and urine.

Images



Western blot - Anti-CD133 antibody - Stem Cell Marker (ab19898)

All lanes: Anti-CD133 antibody - Stem Cell Marker (ab19898) at 1 µg/ml

Lane 1: Caco 2 cell lysate Lane 2: HeLa cell lysate

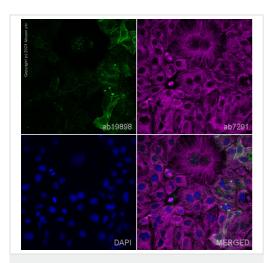
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

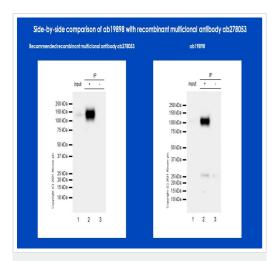
Predicted band size: 97 kDa Observed band size: 97 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab19898 observed at 97 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab19898 was shown to react with CD133 in western blot. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab19898 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye $^{\circledR}$ 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-CD133 antibody - Stem Cell Marker (ab19898)



Immunoprecipitation - Anti-CD133 antibody - Stem Cell Marker (ab19898)

ab19898 staining CD133 in Caco-2 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab19898 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor®594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

Immunoprecipitation side-by-side comparison with the recombinant multiclonal antibody <u>ab278053</u>

This immunoprecipitation image is a comparison between ab19898 and the alternative recombinant multiclonal antibody ab278053.

Left side - Recombinant multiclonal to CD133 - <u>ab278053</u> CD133 was immunoprecipitated from 0.35 mg Caco-2 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate with <u>ab278053</u> at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab278053</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: Caco-2 whole cell lysate 10 µg.

Lane 2: ab278053 IP in Caco-2 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of $\underline{ab278053}$ in Caco-2 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.

Right side - Polyclonal antibody to [CD133] - ab19898

Same testing conditions as ab278053.

Why choose a recombinant antibody?

Research with confidence - consistent and reproducible results with

every batch

Long-term and scalable supply - powered by recombinant technology for fast production

Success from the first experiment - confirmed specificity through extensive validation

Ethical standards compliant - production is animal-free

Western blot - Anti-CD133 antibody - Stem Cell Marker (ab19898)

Western blot side-by-side comparison with the recombinant multiclonal antibody **ab278053**

This Western blot image is a comparison between ab19898 and the alternative recombinant multiclonal antibody **ab278053**.

Left side - Recombinant multiclonal to CD133 - ab278053

All lanes : Anti-CD133 antibody [RM1002] (ab278053) at 1/1000 dilution

Lane 1 : Caco-2 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : HEK-293T (Human embryonic kidney epithelial cell) whole cell lysate

Lane 3 : NCCIT (Human pluripotent embryonic carcinoma epithelial cell) whole cell lysate

Lane 4: HT-29 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

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

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 26 seconds.

Right side - Polyclonal antibody to [CD133] - ab19898

Same testing conditions as ab278053.

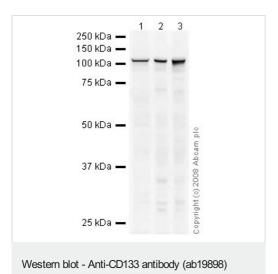
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All lanes : Anti-CD133 antibody - Stem Cell Marker (ab19898) at 1 μ g/ml

Lane 1 : MEL-1 (Human embryonic stem cell, male cell line) Whole Cell Lysate (<u>ab27198</u>)

Lane 2: HT-29 whole cell lysate (ab3952)

Lane 3: Caco-2 whole cell lysate (ab3950)

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 97 kDa **Observed band size:** 110 kDa

ab19898 contains a number of potential glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.

1 2
250kDa — _____
150kDa — ____
100kDa — ____
75kDa — ____
50kDa — ____
37kDa —

Western blot - Anti-CD133 antibody (ab19898)

All lanes: Anti-CD133 antibody - Stem Cell Marker (ab19898) at 1 µg/ml

Lane 1: Human Embryonic Stem Cell Lysate

Lane 2: Human Embryonic Stem Cell Lysate with CD133 peptide (ab20651) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Predicted band size: 97 kDa

Anti-CD133 antibody ab19898 detects a band corresponding to the expected size of CD133 in Human Embryonic Stem Cell Lysate using Western Blotting. It is likely that the band runs higher than the

predicted MW of CD133 due to glycosylation of CD133. In mouse neural stem cell and mouse embryonic stem cell lysate this ~110 kDa band was not detected but a reproducible band of ~30 kDa was seen. This could represent a cleavage product but it may also be that the ~30 kDa band represents non-specific binding by ab19898. However, ab19898 has been shown to recognise mouse neural stem cells using ICC. Blocking using the immunising peptide removed both of these bands.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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