abcam

Product datasheet

Anti-CD133 antibody [EPR16508] ab222782





★★★★★ 1 Abreviews 28 References 8 Images

Overview

Product name Anti-CD133 antibody [EPR16508]

Description Rabbit monoclonal [EPR16508] to CD133

Host species Rabbit

Tested applications Suitable for: WB, IHC-P Species reactivity Reacts with: Human

Predicted to work with: Non human primates

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HAP1, Caco-2 (Human colorectal adenocarcinoma epithelial cell), NCCIT (Human

> pluripotent embryonic carcinoma epithelial cell) and HT-29 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate. Human fetal pancreas and fetal kidney lysate. IHC-P: Human

pancreas and kidney tissue. Human bladder cancer tissue.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol, 59% PBS

Purity Protein A purified

Clonality Monoclonal

Clone number EPR16508

Isotype lgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab222782 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
WB		1/2000. Predicted molecular weight: 97 kDa.
IHC-P	**** (1)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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

lsoform 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 Belongs to the prominin family.

Sequence similarities

Post-translational

Isoform 1 and isoform 2 are glycosylated.

modifications

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

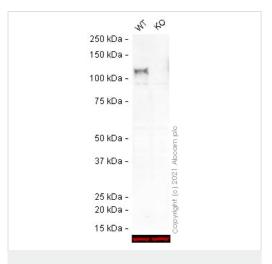
expression and its function in cell apoptosis.

Cellular localization

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.



Western blot - Anti-CD133 antibody [EPR16508] (ab222782)

All lanes : Anti-CD133 antibody [EPR16508] (ab222782) at 1/2000 dilution

Lane 1: Wild-type HAP1 cell lysate

Lane 2: PROM1 knockout HAP1 cell lysate

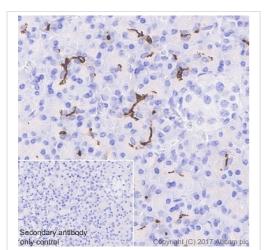
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

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

Exposure time: 8 minutes

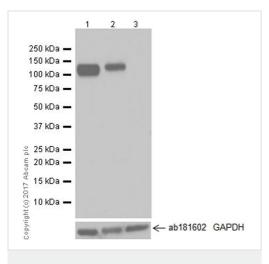
ab222782 was shown to react with CD133 in wild-type HAP1 cells in western blot. Loss of signal was observed when PROM1 knockout sample was used. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab222782 overnight at 4°C at a 1 in 2000 dilution. Blots were incubated with HRP conjugated Goat anti-Rabbit (H+L) secondary antibody at 1/5000 for 1 hour at room temperature before development with ECL reagent and imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD133 antibody
[EPR16508] (ab222782)

Immunohistochemical analysis of paraffin-embedded human pancreas tissue labeling CD133 with ab222782 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP)(ready use). Apical/endoluminal staining of human pancreatic ducts, and negative on Langerhans cells of the islets (PMID: 18261235). Counter stained with Hematoxylin. Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ready to use).



Western blot - Anti-CD133 antibody [EPR16508] (ab222782)

All lanes : Anti-CD133 antibody [EPR16508] (ab222782) at 1/10000 dilution

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

Lane 2: HT-29 (Human colorectal adenocarcinoma epithelial cell), whole cell lysate

Lane 3: HeLa (Human cervix adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 97 kDa

Exposure time: 5 seconds

Negative control: HeLa (PMID 9389721).

The MW observed is consistent with the literature (PMID 20717901; PMID 18645205).

Blocking buffer: 5% NFDM/TBST.

250 kDa — 150 kDa — 100 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kDa —

1

Western blot - Anti-CD133 antibody [EPR16508] (ab222782)

Anti-CD133 antibody [EPR16508] (ab222782) at 1/10000 dilution + NCCIT (Human pluripotent embryonic carcinoma epithelial cell), whole cell lysate at 20 μ g

Secondary

Goat Anti-Rabbit lgG H&L (HRP) ($\underline{ab97051}$) at 1/100000 dilution

Predicted band size: 97 kDa

Exposure time: 15 seconds

Blocking buffer: 5% NFDM/TBST.

The MW observed is consistent with the literature (PMID 20717901;

1 2
250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

Western blot - Anti-CD133 antibody [EPR16508] (ab222782)

PMID 18645205).

All lanes : Anti-CD133 antibody [EPR16508] (ab222782) at 1/2000 dilution

Lane 1 : Human fetal pancreas lysate

Lane 2 : Human fetal kidney 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

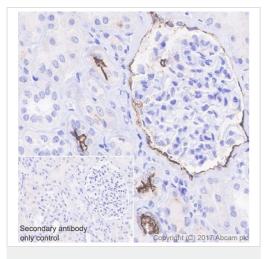
Exposure time: 3 minutes

Blocking buffer: 5% NFDM/TBST.

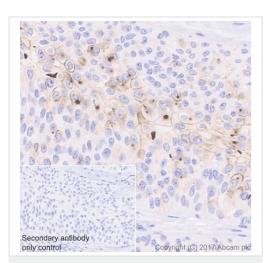
The MW observed is consistent with the literature (PMID 20717901; PMID 18645205).

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling CD133 with ab222782 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ready to use). Membranous staining on parietal layer of Bowman's capsule and apical staining on renal tubules in human kidney (PMID: 19092120). Counter stained with Hematoxylin. Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ready to use).



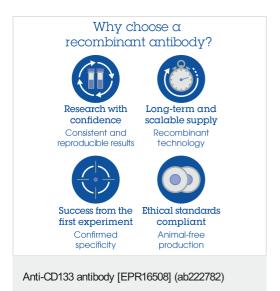
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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD133 antibody
[EPR16508] (ab222782)

Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling CD133 with ab222782 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ready to use). Membranous staining on human bladder cancer tumor cells (PMID: 22899341). Counter stained with Hematoxylin. Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ready to use).



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