

ANTI- PROMININ-1(CD133) MONOCLONAL ANTIBODY

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|-------------------------|---------------------|-----------------------|------------------------------|
| CATALOG NUMBER: | MAB4310 | QUANTITY: | 100 µg |
| LOT NUMBER: | | | |
| ALTERNATE NAMES: | CD133; AC133(human) | CONCENTRATION: | 1.0 mg/mL |
| CLONE NAME: | 13A4 | HOST/ISOTYPE: | Rat / IgG ₁ kappa |

BACKGROUND: Prominin is a novel plasma membrane protein with an N-terminal extracellular domain, five transmembrane segments flanking two short cytoplasmic loops and two large glycosylated extracellular domains, and a cytoplasmic C-terminal domain. DNA sequences from *Caenorhabditis elegans* predict the existence of a protein with the same features, suggesting that prominin is conserved between vertebrates and invertebrates. Prominin is found not only in the neuroepithelium but also in various other epithelia of the mouse embryo. In the adult mouse, prominin has been detected in the brain ependymal layer, and in kidney tubules. In these epithelia, prominin is specific to the apical surface, where it is selectively associated with microvilli and microvilli-related structures. Remarkably, upon expression in CHO cells, prominin is preferentially localized to plasma membrane protrusions such as filopodia, lamellipodia, and microspikes. These observations imply that prominin contains information to be targeted to, and/or retained in, plasma membrane protrusions rather than the planar cell surface. Moreover, results show that the mechanisms underlying targeting of membrane proteins to microvilli of epithelial cells and to plasma membrane protrusions of non-epithelial cells are highly related. {Weigmann, A et al.1997}. Also see {http://www.ncbi.nlm.nih.gov/prow/guide/438375806_g.htm} for more information on CD133 and prominin expression.

SPECIFICITY: The 13A4 monoclonal antibody recognizes mouse Prominin-1 (sometimes also referred to as CD133 and, in the case of the human orthologue, as AC133), a 115-120 kDa pentaspan transmembrane (5-TM) domain glycoprotein. Prominin-1 is expressed on primitive cells such as hematopoietic stem and progenitor cells, neural & endothelial stem cells, retina and retinoblastoma, as well as developing epithelium. To date, the function and ligand of Prominin-1 are unknown.
 This antibody can be used to isolate murine stem cells from brain and bone marrow. The 13A4 antibody does not cross react with rat, human, chicken, or Drosophila antigen. The original paper is located at: <http://www.pnas.org/cgi/reprint/94/23/12425.pdf>

IMMUNOGEN: Telencephali of ten 12-day-old NMRI mouse embryos (E12, ~1 mg protein), which consist mostly of neuroepithelium, were homogenized in 1 ml PBS, mixed with crushed nitrocellulose filter (1 cm² in 0.5 ml PBS) as adjuvant, and injected i.p. into a LouXSD rat. {Weigmann et al, PNAS 1997}

APPLICATIONS: Immunohistochemistry: Recommended antibody dilution: 1-15 µg/ml
 Suggested fixatives: 2-4% PFA, or methanol -20°C fixation. When fixing cells in culture, incubate sample in 3% PFA for 15-30 min at room temperature. Tissue: perfusion with 2-4% PFA, 1-4 hours postfixation is typical. Traditional formalin fixation is NOT recommended.
 Permeabilization method: 0.2% saponin or 0.1-0.3% Triton X-100 in PBS.

Blocking Buffer: For cells in culture, 0.2% gelatin in PBS; for tissue section, 10% FCS in PBS.

Dilution Buffer: For cells in culture, 0.2% gelatin in PBS; for tissue section, 0.2% saponin and 10% FCS in PBS.

Incubation Times/Temperature: Overnight at 4°C or 1 hour at 37°C

Recommended control tissue: Positive: Kidney proximal tubules or epididymis; Negative: liver

Note: This antibody does not work with paraffin-embedded sections.

EM immunohistochemistry: The subcellular localization of the 13A4 antigen in mouse E9–10 neuroepithelial cells and adult kidney proximal tubule cells was investigated by immunogold electron microscopy. Strong labeling was observed over the kidney brush border membrane, where 13A4 immunoreactivity appeared to be concentrated toward the tips of the microvilli. Remarkably, in neuroepithelial cells, whose apical plasma membrane contains fewer microvilli than the kidney brush border, 13A4 immunoreactivity was associated mostly, if not exclusively, with microvilli and plasma membrane protrusions, and was not detected in the planar areas of the apical plasma membrane. Because of this preferential localization, we propose to call the 13A4 antigen “prominin” (from the Latin word “prominere,” to stand out, to be prominent).
{<http://www.pnas.org/cgi/reprint/94/23/12425.pdf>}.

Western blotting: Recommended primary antibody dilution: 1-5 µg/ml in 0.3% Tween in PBS. Sample preparation: Standard Laemmli (SDS, reducing agents, boiled {2% SDS, 100mM DTT or 5% beta-mercaptoethanol, 60mM Tris-HCL pH 6.8} Preferred Gel percentage: 7.5%. Suggested Blocking Buffer: 3-5% Milk, 0.3% Tween in PBS. Incubation time: 1 hour at room temperature or overnight at 4°C.

Prominin is a membrane-associated 115-kDa glycoprotein. Immunoblotting of E12 mouse brain and adult mouse kidney using mAb 13A4 showed that prominin has a mean apparent molecular weight of 115 kDa; Deglycosylation of brain and kidney prominin with peptide N-glycosidase F (PNGase F) yielded a 94-kDa band indicating that prominin is N-glycosylated. (The additional 88-kDa band seen upon deglycosylation of brain prominin represents a C-terminally truncated form {Weigmann et al <http://www.pnas.org/cgi/reprint/94/23/12425.pdf>} also see {Maw, 2000 <http://hmg.oupjournals.org/cgi/content/full/9/1/27> or http://www.nature.com/ncb/journal/v2/n9/extref/ncb0900_582-s1.pdf})

Recommended control extracts: Positive: Kidney membrane; Negative: liver membranes.

Immunoprecipitation: Suggested antibody dilution/amount: 10-25 µg/ml. Suggested tissue/cell lysis buffer: RIPA buffer Final reaction volume: 500-1000 µl. Final total protein concentration in reaction mix: 0.5-3 mg/mL. Incubation times: overnight at 4°C. Capture agent used: Protein G sepharose or rabbit anti-rat antibody/protein A sepharose. Expected sizes on immunoblots (in kDa): 115 kDa (mature form) or 105 kDa (precursor form).

FACS Analysis: Suggested dilution/number of cells: 0.25 -1 µg/ million cells.

Fixation/Permeabilization used: BD FACSTM lysis Solution (1-1.5% formaldehyde) {BD and FACSTM are trademarks of Becton, Dickinson and Company} No permeabilization.

Recommended controls: Hematopoietic stem cells

Optimal working dilutions must be determined by end user.



- SPECIES REACTIVITY:** Reacts with mouse; does not react with human, rat, chicken or *Drosophila*.
- CONTROL:** Positive control: Kidney tissue or membranes; Negative control: Liver tissue or membranes
- PRESENTATION:** Purified immunoglobulin in 0.02M PB with 0.25M NaCl, pH 7.6. Contains 0.1% sodium azide
- STORAGE/HANDLING:** Maintain refrigerated at 2-8°C in undiluted aliquots for up to 6 months after date of receipt.
- REFERENCES:**
- Weigmann, A et al. (1997). Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells {original paper}. PNAS, USA 94:12425-12430.
- Related: Sawamoto, K et al. (2001). Generation of Dopaminergic Neurons in the Adult Brain from Mesencephalic Precursor Cells Labeled with a nestin-GFP Transgene . J Neuroscience 21(11):3895-3903.
- Related: Roper K et al (2000). Retention of prominin in microvilli reveals distinct cholesterol-based lipid micro-domains in the apical plasma membrane. Nature Cell Biol 2(9):582-592.
- Related: Corbeil D. et al. (1998). AC133 Hematopoietic Stem Cell Antigen: Human Homologue of Mouse Kidney Prominin or Distinct Member of a Novel Protein Family?. Blood 91(7):2625-2626.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μ L or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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