

Anti-Prominin-1-PE	130-092-334
Anti-Prominin-1-APC	130-092-335
Anti-Prominin-1-Biotin	130-092-441
Anti-Prominin-1 pure	130-092-442

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1. Description

Clone	MB9-3G8 (isotype: rat IgG1).
Product format	1 mL Anti-Prominin-1 antibody, mouse: monoclonal Anti-Prominin-1 antibodies conjugated to R-phycoerythrin (PE), or allophycocyanin (APC), or biotin (Biotin). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	100 tests or up to 10 ⁹ total cells.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The Anti-Prominin-1 antibody has been developed for the detection of mouse cells expressing prominin-1. Prominin-1 is a 115–120 kDa, 5-transmembrane glycoprotein with close structural similarity to its human ortholog, CD133. Prominin-1 is expressed on neural stem cells, for example in the white matter of the cerebellum¹, neuroepithelial tissue and various other epithelia in the mouse embryo². Ten to fifteen percent of the neural cells in the subventricular zone of postnatal mice were shown to be prominin-1⁺. In the adult mouse, prominin-1 has been detected in the brain ependymal layer², and on kidney tubules².

Product applications

- Identification and enumeration of prominin-1⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy. Mouse prominin-1⁺ cells can be isolated by using Anti-Prominin-1 MicroBeads, mouse (# 130-092-333).

1.2 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-Prominin-1 conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11
- Anti-Prominin-1 MicroBead-labeled cells	1:11	1:11	1:11
Immunohistochemistry^c			
a) Given antibody dilutions are for a cell concentration of up to 10 ⁷ cells/100 µL of buffer.			
b) For optimal results, cells must be stained prior to fixation.			
c) The optimal antibody dilution should be determined.			

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA, e.g. by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C).
▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse, to avoid Fc receptor-mediated fluorescent staining.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-Prominin-1-Biotin.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

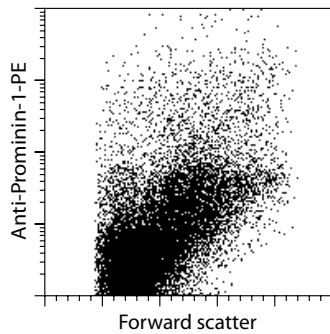
1. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
▲ **Note:** (Optional) if FcR Blocking Reagent, mouse, is being used, resuspend 10⁷ nucleated cells in 90 µL of buffer and add 10 µL of FcR Blocking Reagent, mouse directly before addition of the Anti-Prominin-1 antibody.
2. Add 10 µL of Anti-Prominin-1 antibody.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °C).
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

5. (Optional) If Anti-Prominin-1-Biotin was used, resuspend the cell pellet in 100 μ L buffer, add 10 μ L Anti-Biotin antibody (Anti-Biotin-FITC #130-090-857, Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), and continue as described in steps 3 and 4.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

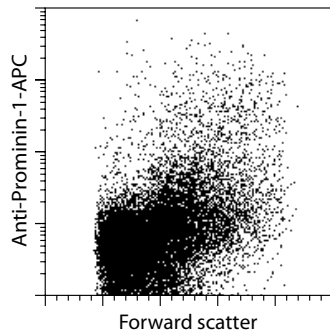
3. Examples of immunofluorescent staining with Anti-Prominin-1 antibodies

Neural cells from the cerebella of P1-mice were prepared from freshly resected brain tissue using the Neural Tissue Dissociation Kit (# 130-092-628). Neural cells were stained with Anti-Prominin-1 antibodies conjugated to PE (a), or APC (b), or Biotin (c), and analyzed by flow cytometry. For Anti-Prominin-1-Biotin, Anti-Biotin-APC (# 130-090-856) was used as secondary antibody. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

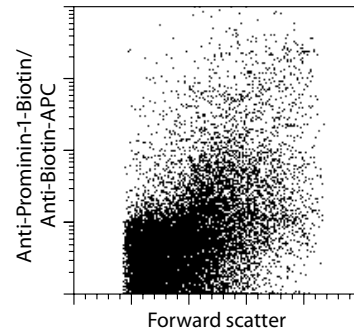
- (a) Neural cells from the cerebella of P1-mice stained with Anti-Prominin-1-PE.



- (b) Neural cells from the cerebella of P1-mice stained with Anti-Prominin-1-APC.



- (c) Neural cells from the cerebella of P1-mice stained with Anti-Prominin-1-Biotin and Anti-Biotin-APC.



4. References

1. Lee, A. *et al.* (2005) Isolation of neural stem cells from the postnatal cerebellum. *Nat. Neurosci.* 8: 723–729.
2. 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. *Proc. Natl. Acad. Sci, USA* 94: 12425–12430.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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