



Anti-PSA-NCAM antibodies

human, mouse, rat

Anti-PSA-NCAM-PE
Anti-PSA-NCAM-APC

130-093-274
130-093-273

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1. Description

Components	1 mL Anti-PSA-NCAM antibodies, human, mouse, rat; monoclonal Anti-PSA-NCAM antibodies conjugated to R-phycoerythrin (PE), or allophycocyanin (APC).
Clone	2-2B (isotype: mouse IgM).
Capacity	100 tests or up to 10 ⁸ total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The Anti-PSA-NCAM antibody recognizes an epitope on human, mouse, and rat polysialic acid (PSA) which, in vertebrates, is found linked to the extracellular domain of the neural cell adhesion molecule (NCAM, CD56)¹. PSA-NCAM, the highly polysialated form of NCAM, is predominantly expressed in embryonic and neonatal neural tissue.² In adult mammalian brain PSA-NCAM expression is restricted mainly to areas that retain neurogenic potential, such as the subventricular zone (SVZ)³ and the dentate gyrus of the hippocampus⁴.

PSA-NCAM is a marker for immature neuronal-committed progenitors that are permanently generated in the SVZ and migrate along a well-defined pathway, the rostral migratory stream, into the olfactory bulb where they differentiate into GABAergic and dopaminergic interneurons.^{3,5}

Antibodies against PSA-NCAM have also been used to immunomagnetically isolate neuronal progenitors from postnatal mice and rat forebrain.⁶⁻⁸

1.2 Applications

- Evaluation of MACS® Separations by flow cytometry. Mouse neuronal precursors can be isolated by using, for example, Anti-PSA-NCAM MicroBeads, human, mouse, rat (# 130-092-966).

1.3 Recommended antibody dilution

For antibody labeling of human, mouse, and rat cells.

Anti-PSA-NCAM conjugate	PE	APC
Flow cytometry^a		
- In general	1:11	1:11
- Anti-PSA-NCAM MicroBead-labeled cells	1:11	1:11
a) The indicated antibody dilutions are for up to 10 ⁶ cells/100 µL of buffer.		

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °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 human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human or mouse (# 130-059-901) and # 130-092-575, respectively) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.
- (Optional) Neural Tissue Dissociation Kit (T) (# 130-093-231) for dissociation of brain tissue.
- (Optional) gentleMACS™ Dissociator.

2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ 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 2×10⁶ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

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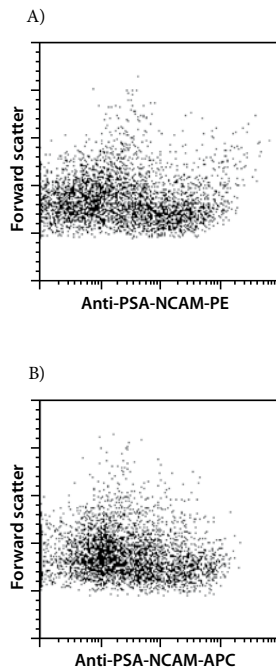
- Resuspend up to 10^6 nucleated cells per 100 μL of buffer.
- Add 10 μL of the Anti-PSA-NCAM antibody.
- Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

- Wash cells by adding 1–2 mL of buffer and centrifuge at $300\times g$ for 10 minutes. Aspirate supernatant completely.
- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

3. Examples of immunofluorescent staining with Anti-PSA-NCAM antibodies

Mouse brain tissue postnatal day 1 was dissociated using the Neural Tissue Dissociation Kit (T). Brain cells were stained with Anti-PSA-NCAM antibody conjugated to PE (A) or APC (B) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

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All protocols and data sheets are available at www.miltenyibiotec.com.

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.

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