

# Anti-A2B5 antibodies

## human, mouse, rat

Anti-A2B5-PE	130-093-581
Anti-A2B5-APC	130-093-582
Anti-A2B5-Biotin	130-093-393
Anti-A2B5 pure	130-093-394

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## 1. Description

<b>Components</b>	1 mL Anti-A2B5 antibodies, human, mouse, rat: monoclonal Anti-A2B5 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
<b>Clone</b>	105-HB29 (isotype: mouse IgM).
<b>Capacity</b>	100 tests or up to 10 <sup>8</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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 clone 105-HB29 recognizes the c-series ganglioside-specific antigen A2B5. The Anti-A2B5 antibody binds to glial progenitors from embryonic to adult human, murine, and rat tissue.<sup>1</sup> A2B5 is predominantly expressed in embryonic and neonatal neural tissue. In the adult mammalian brain, A2B5 expression is restricted mainly to areas which retain neurogenic potential such as the subventricular zone (SVZ). Thus, A2B5 is considered as a marker for immature glial-committed precursors which are permanently generated in the SVZ. Glial precursor cells are defined as cells that give rise to glial cell types, such as astrocytes and oligodendrocytes. Ganglioside GT3 and its O-acetylated derivative are the principal A2B5-reactive gangliosides<sup>2</sup>, and both antigens are down-regulated as the cells differentiate into mature oligodendrocytes. Therefore, A2B5 serves as a marker to monitor the maturation of oligodendrocyte progenitors in oligodendrocyte cultures.

### 1.2 Applications

- Identification of A2B5<sup>+</sup> glial precursor cells by flow cytometry or fluorescence microscopy.<sup>3-7</sup> The Anti-A2B5 antibody can be used in oligodendrocyte cultures to monitor the maturation of oligodendrocyte progenitors.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human, mouse, or rat glial precursors can be isolated by using, for example, Anti-A2B5 MicroBeads (# 130-093-388).

### 1.3 Recommended antibody dilution

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

Anti-A2B5 conjugate	PE	APC	Biotin
<b>Flow cytometry<sup>a</sup></b>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11
- Anti-A2B5 MicroBead-labeled cells	1:11	1:11	1:11
<b>Immunohistochemistry<sup>c</sup></b>			
a) The indicated antibody dilutions are for up to 10 <sup>6</sup> cells/100 µL of buffer. b) Cells must be stained prior to fixation. c) The optimal antibody dilution should be determined by the user.			

### 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> 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) 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-A2B5-Biotin.
- (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) or Neural Tissue Dissociation Kit (P) (# 130-092-628) for dissociation of brain tissue.

- (Optional) gentleMACS™ Dissociator.

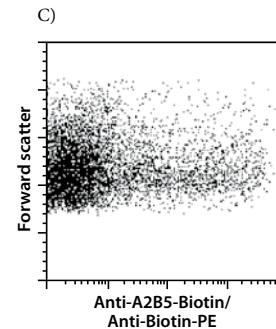
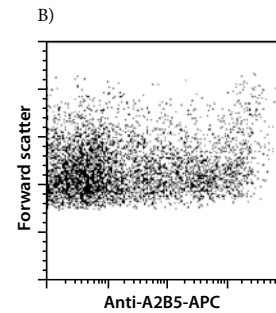
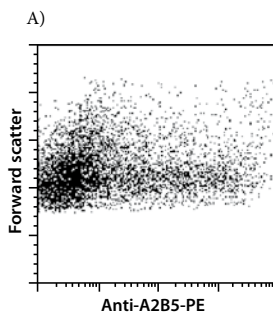
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^6$  nucleated cells. When working with fewer than  $10^6$  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  $2 \times 10^6$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^6$  nucleated cells per 100  $\mu$ L of buffer.
4. Add 10  $\mu$ L of the Anti-A2B5 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ \text{C}$ ).  
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-A2B5-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

## 3. Examples of immunofluorescent staining with Anti-A2B5 antibodies

Mouse brain tissue postnatal day 1 was dissociated using the Neural Tissue Dissociation Kit (T). Brain cells were stained with Anti-A2B5 antibodies conjugated to PE (A), APC (B), or Biotin (C) and analyzed by flow cytometry. Cells labeled with Anti-A2B5-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. References

1. Dietrich, J. *et al.* (2002) Characterization of A2B5+ glial precursor cells from cryopreserved human fetal brain progenitor cells. *Glia* 40: 65–77.
2. Dubois, C. *et al.* (1990) Monoclonal antibody A2B5, which detects cell surface antigens, binds to ganglioside GT3 (II3 (NeuAc)3LacCer) and to its 9-O-acetylated derivative. *J. Biol. Chem.* 265: 2797–2803.
3. Larsen, P. H. *et al.* (2003) Matrix metalloproteinase-9 facilitates remyelination in part by processing the inhibitory NG2 proteoglycan. *J. Neurosci.* 23: 11127–11135.
4. Ruffini, F. *et al.* (2004) Distinctive properties of human adult brain-derived myelin progenitor cells. *Am. J. Pathol.* 165: 2167–2175.
5. Windrem, M. S. (2004) Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nat. Med.* 10: 93–97. Erratum in: *Nat. Med.* (2004) 10: 209.
6. Larsen, P. H. and Yong, V. W. (2004) The expression of matrix metalloproteinase-12 by oligodendrocytes regulates their maturation and morphological differentiation. *J. Neurosci.* 24: 7597–7603.
7. Larsen, P. H. *et al.* (2006) Myelin formation during development of the CNS is delayed in matrix metalloproteinase-9 and -12 null mice. *J. Neurosci.* 26: 2207–2214.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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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