Anti-Plexin-B2 antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
Anti-Plexin-B2-PE	for 30 tests	130-109-771
Anti-Plexin-B2-PE	for 100 tests	130-109-724
Anti-Plexin-B2-APC	for 30 tests	130-109-772
Anti-Plexin-B2-APC	for 100 tests	130-109-725
Anti-Plexin-B2-PE-Vio770	for 30 tests	130-109-773
Anti-Plexin-B2-PE-Vio770	for 100 tests	130-109-726

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.

Technical data and background information

Antigen	Plexin-B2
Clone	REA626
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	Plxnb2, MM1, Nbla00445, dJ402G11.3
Molecular mass of antigen [kDa]	203
Distribution of antigen	dendritic cells, macrophages, T cells, astrocytes, microglia, neurons, oligodendrocytes, other
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA626 recognizes the human plexin-B2 antigen, a single-pass type I membrane protein, which belongs to the semaphorin receptor family, B subfamily. Plexin-B2 is expressed in the nervous system and on other cells including cells of the immune system. It is expressed on T cells and T cell–dependent germinal center B cells. Expression is also found on macrophages, conventional dendritic cells, and plasmacytoid dendritic cells. Plexin-B2 plays an important role in cell-cell signaling, axon guidance, invasive growth, and cell migration. It has several semaphorin ligands including semaphorin-3E, semaphorin-4A, semaphorin-4C, and semaphorin-4D (CD100). Binding to class 4 semaphorins promotes the downstream activation of RHOA and phosphorylation of ERBB2. During

skin damage repairs, CD100 interacts with plexin-B2 on γδ T cells to play a role in the healing process. Additional information: Clone REA626 displays negligible binding to Fc receptors.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

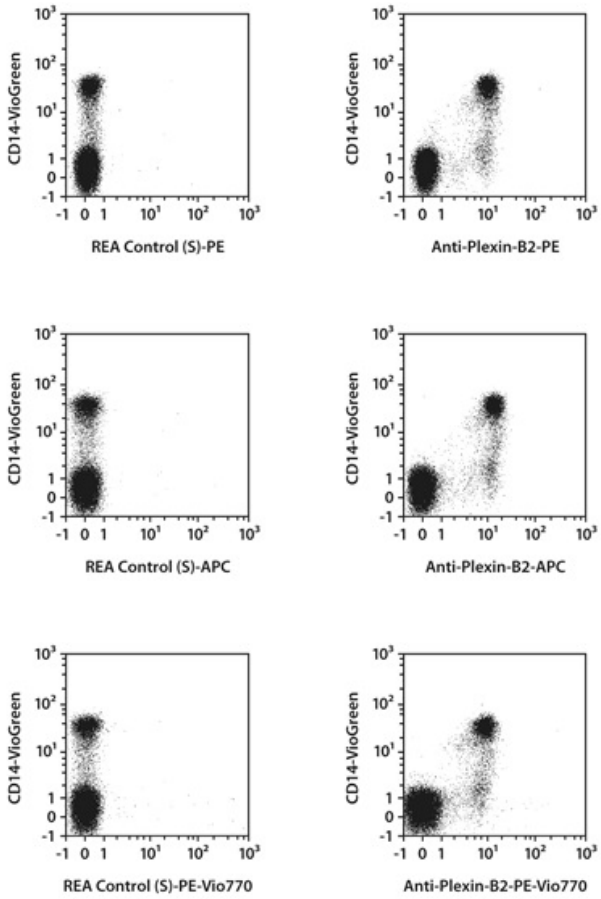
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 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 2×10^7 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.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, 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.

Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-Plexin-B2 antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD14 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



References

1. **Deng, S. *et al.*** (2007) Plexin-B2, but not Plexin-B1, critically modulates neuronal migration and patterning of the developing nervous system *in vivo*. J. Neurosci. 27(23): 6333–6347.
2. **Witherden, D.A. *et al.*** (2012) The CD100 receptor interacts with its plexin B2 ligand to regulate epidermal γδ T cell function. Immunity 37(2): 314–325.
3. **Le, A. P. *et al.*** (2015) Plexin-B2 promotes invasive growth of malignant glioma. Oncotarget 6(9): 7293–7304.

Warranty

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