Anti-Plexin-D1 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-D1-VioBright FITC	for 30 tests	130-108-321
Anti-Plexin-D1-VioBright FITC	for 100 tests	130-108-298
Anti-Plexin-D1-PE	for 30 tests	130-108-319
Anti-Plexin-D1-PE	for 100 tests	130-108-296
Anti-Plexin-D1-APC	for 30 tests	130-108-320
Anti-Plexin-D1-APC	for 100 tests	130-108-297
Anti-Plexin-D1-Biotin	for 30 tests	130-108-318
Anti-Plexin-D1-Biotin	for 100 tests	130-108-295

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-D1
Clone	REA542
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	PLXND1, PLEXD1
Molecular mass of antigen [kDa]	208
Distribution of antigen	endothelial cells, 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 REA542 recognizes the human plexin-D1 antigen, a type I transmembrane glycoprotein that is a member of the D subfamily of semaphorin receptors. Plexin-D1 is amongst others the receptor for semaphorin 3E (Sema3E) which is a secreted molecule implicated in axonal path finding and inhibition of developmental and postischemic angiogenesis. Plexin-D1 plays an important role in cell-cell signaling and in regulating the migration of a wide spectrum of cell types, and is able to trigger R-Ras inactivation, leading to axonal and cell repulsion *in vitro*. Moreover, it was shown that Sema3E mediates either axonal attraction or repulsion in distinct neuronal populations, depending on the coexpression of

neuropilin-1 with plexin-D1. Plexin-D1 expression is generally low in normal adult tissues, it is elevated in endothelial cells of tumor vessels and in cancer cells.
Additional information: Clone REA542 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.

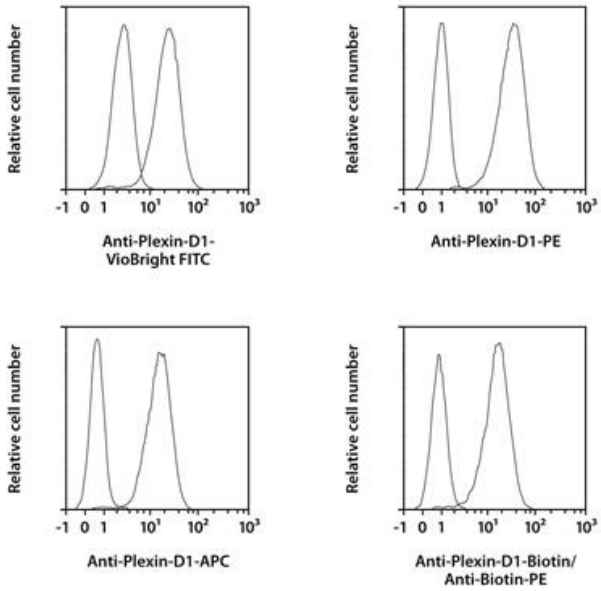
Protocol for cell surface staining

- 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 umbilical vein endothelial cells (HUVEC) were stained with Anti-Plexin-D1 antibodies or with the corresponding REA Control (S) antibodies (left peak). Flow cytometry was performed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **Tamagnone, L. *et al.*** (1999) Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell 99(1): 71–80.
2. **Sakurai, A. *et al.*** (2010) Semaphorin 3E initiates antiangiogenic signaling through plexin D1 by regulating Arf6 and R-Ras. Mol. Cell. Biol. 30(12): 3086–3098.
3. **Casazza, A. *et al.*** (2010) Sema3E–Plexin D1 signaling drives human cancer cell invasiveness and metastatic spreading in mice. J. Clin. Invest. 120(8): 2684–2698.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | **www.miltenyibiotec.com**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in various countries worldwide. Copyright © 2018 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved.