CD317 (PDCA-1) antibodies, mouse

For research use only

30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of 10 cells.

Product	Content	Order no.
CD317 (PDCA-1)-Biotin	30 µg in 200 µL	130-112-375
CD317 (PDCA-1)-FITC	30 µg in 200 µL	130-112-376
CD317 (PDCA-1)-FITC	150 µg in 1 mL	130-112-219
CD317 (PDCA-1)-PE	30 µg in 200 µL	130-112-377
CD317 (PDCA-1)-PE	150 µg in 1 mL	130-112-220
CD317 (PDCA-1)-APC	30 µg in 200 µL	130-112-378
CD317 (PDCA-1)-APC	150 µg in 1 mL	130-112-221
CD317 (PDCA-1)-PE-Vio615	30 µg in 200 µL	130-112-383
CD317 (PDCA-1)-PE-Vio615	150 µg in 1 mL	130-112-226
CD317 (PDCA-1)-PE-Vio770	30 µg in 200 µL	130-112-379
CD317 (PDCA-1)-PE-Vio770	150 µg in 1 mL	130-112-222
CD317 (PDCA-1)-APC-Vio770	30 µg in 200 µL	130-112-380
CD317 (PDCA-1)-APC-Vio770	150 µg in 1 mL	130-112-223
CD317 (PDCA-1)-PerCP-Vio700	30 µg in 200 µL	130-112-381
CD317 (PDCA-1)-PerCP-Vio700	150 µg in 1 mL	130-112-224
CD317 (PDCA-1)-Biotin	150 µg in 1 mL	130-112-218

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	CD317 (PDCA-1)
Clone	REA818
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	BST2, Bst-2, C87040, CD317, DAMP-1, GREG, mPDCA-1
Entrez Gene ID	69550
Molecular mass of antigen [kDa]	17
Distribution of antigen	dendritic cells
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 REA818 recognizes the mouse CD317 antigen, also known as mouse plasmacytoid dendritic cell antigen-1 (PDCA-1) or BST2. CD317 is specifically expressed on mouse PDCs, a subset of $CD11c^{+}$ dendritic cells detected at low frequency in all lymphoid tissues, peripheral blood, and some non-lymphoid tissues. In mouse spleen, bone marrow, and lymph nodes, CD317 is exclusively expressed on cells which are $CD11c^{low}$, $Ly\text{-}6C^{high}$, $CD45R\ (B220)^{+}$, MHC class II^{low}, $CD8a^{low/-}$, $CD80^{low/-}$, $CD86^{-}$, $CD40^{-}$, $CD11b^{-}$, $CD90^{-}$, $CD49b\ (DX5)^{-}$, $CD3^{-}$, $CD19^{-}$, i.e., on cells with the phenotype of mouse PDCs. Multi-color fluorescent staining of spleen cells clearly revealed that all $CD11c^{low}$ $Ly\text{-}6C^{high}$ $CD45R\ (B220)^{+}$ PDCs are $PDCA\text{-}1^{+}$ and that PDCA-1 expression is restricted to PDCs. Additional information: Clone REA818 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.

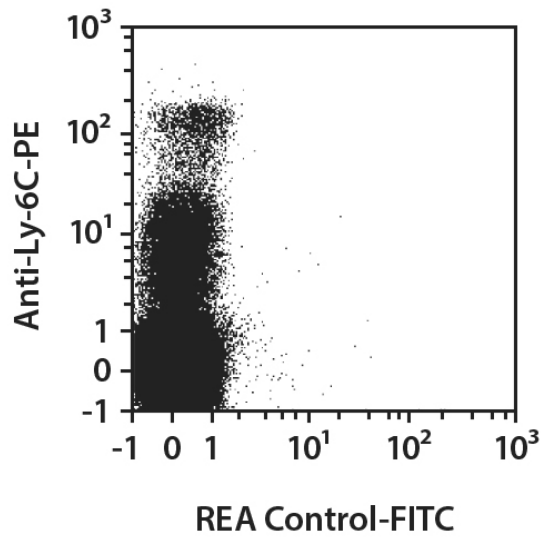
Protocol for cell surface staining

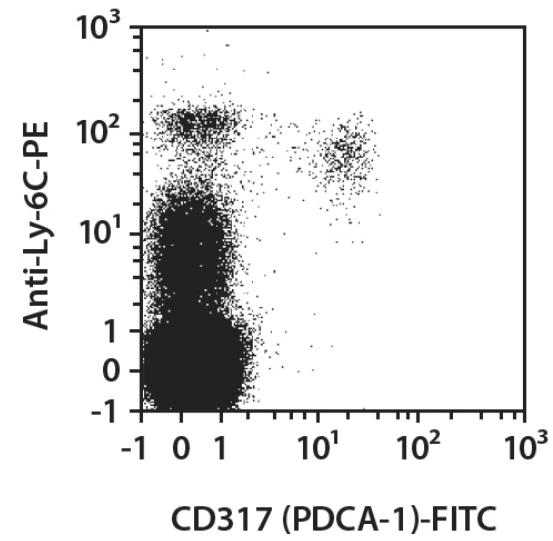
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^{6} cells/100 µL.
- Volumes given below are for up to 10^{6} nucleated cells. When working with fewer than 10^{6} cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

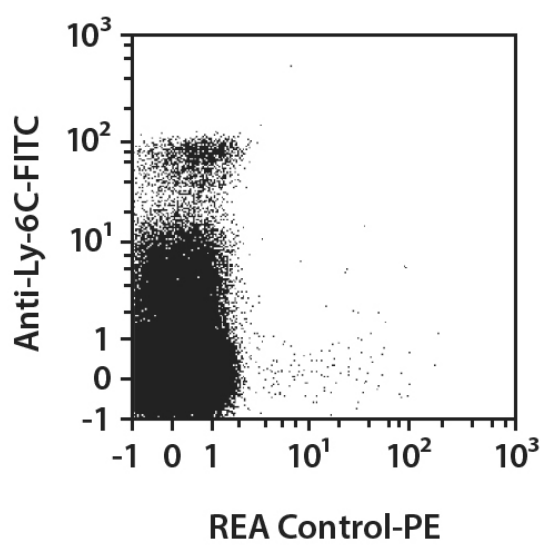
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^{6} nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

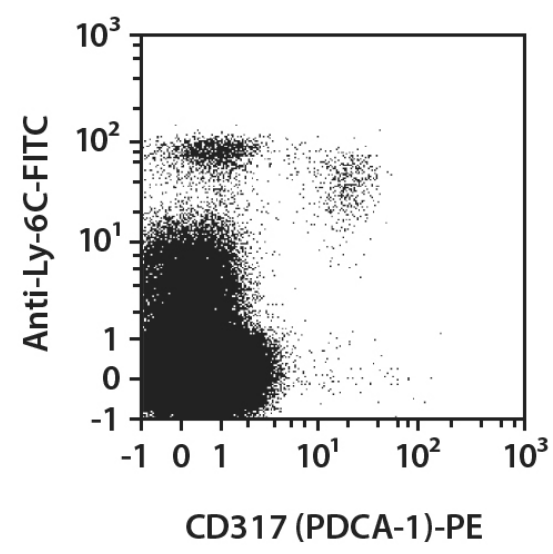
Examples of immunofluorescent staining

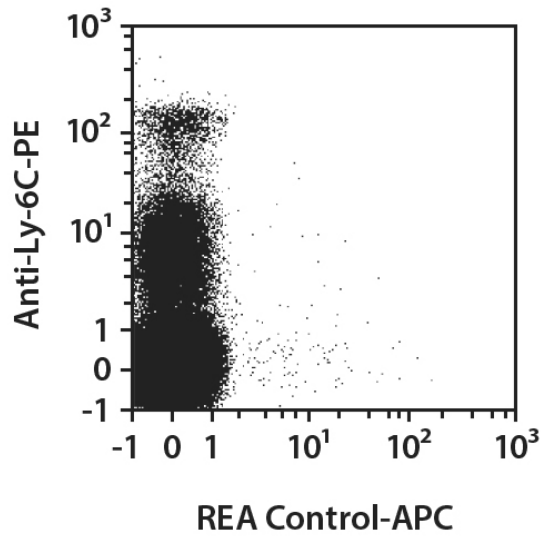
Splenocytes of BALB/c mice were stained with CD317 (PDCA-1) antibodies or with the corresponding REA Control antibodies (left image) as well as with Anti-Ly-6C antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

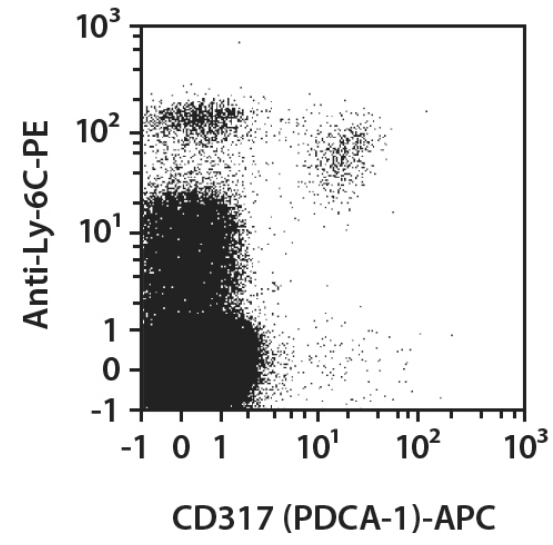

Anti-Ly-6C-PE
REA Control-FITC

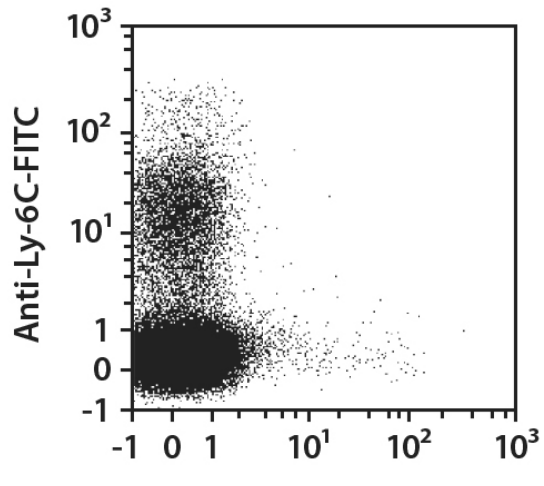

Anti-Ly-6C-PE
CD317 (PDCA-1)-FITC

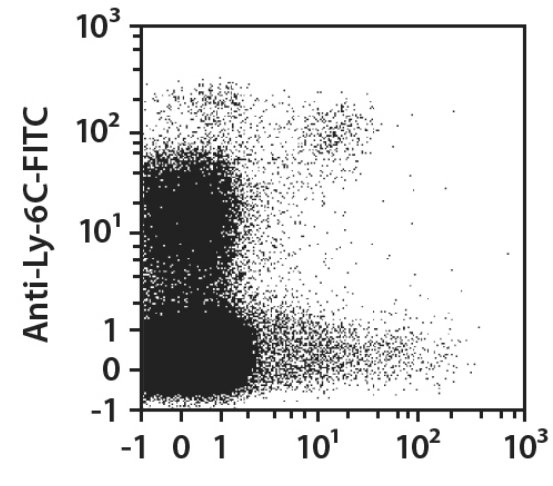

Anti-Ly-6C-FITC
REA Control-PE

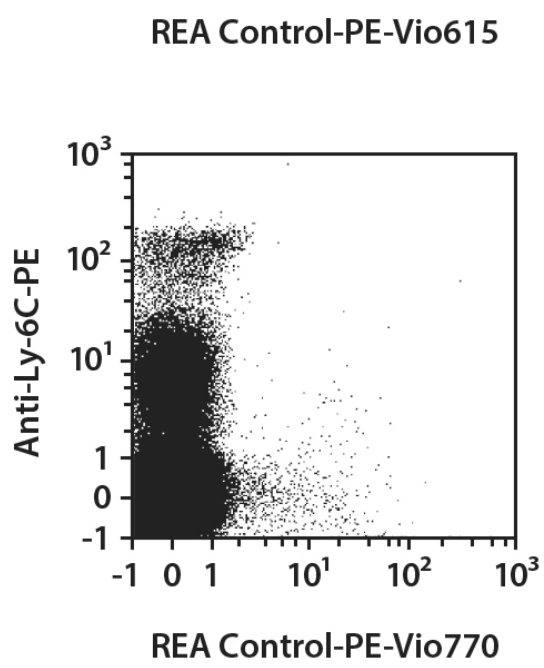

Anti-Ly-6C-FITC
CD317 (PDCA-1)-PE

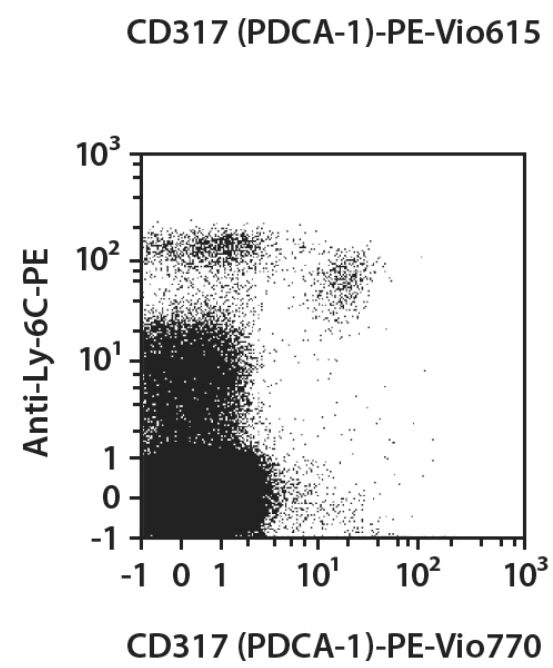

Anti-Ly-6C-PE
REA Control-APC

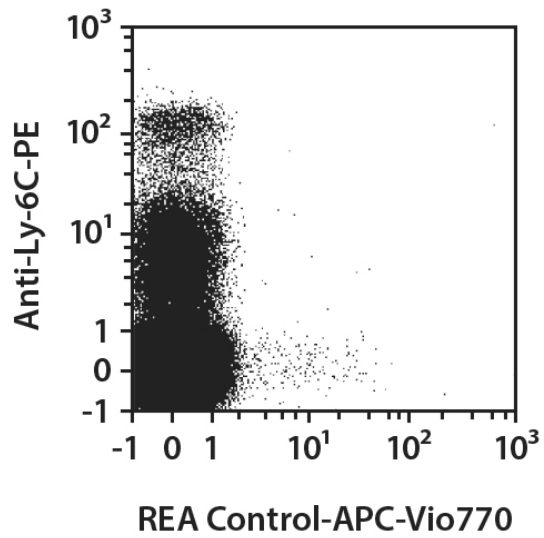

Anti-Ly-6C-PE
CD317 (PDCA-1)-APC

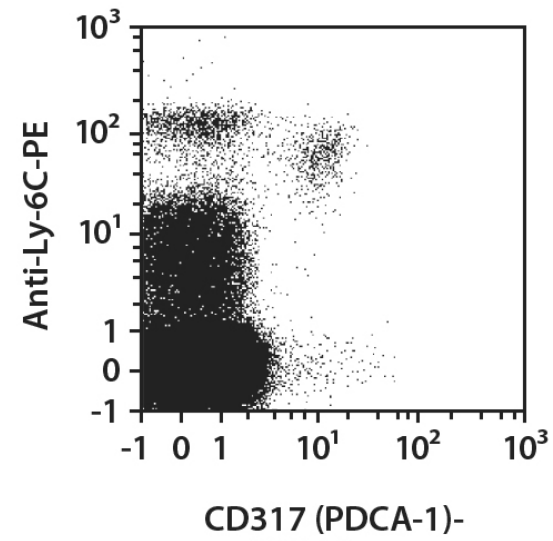

Anti-Ly-6C-FITC
REA Control-PE-Vio615

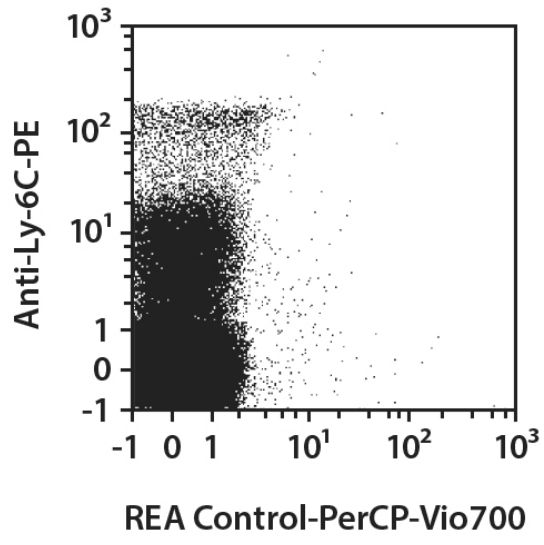

Anti-Ly-6C-FITC
CD317 (PDCA-1)-PE-Vio615

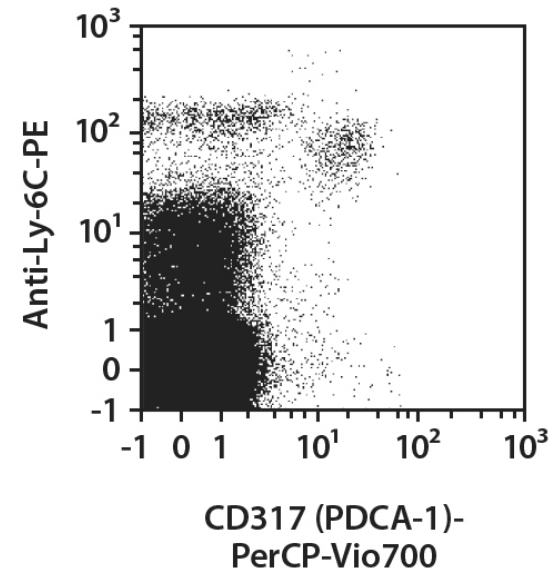

Anti-Ly-6C-PE
REA Control-PE-Vio770

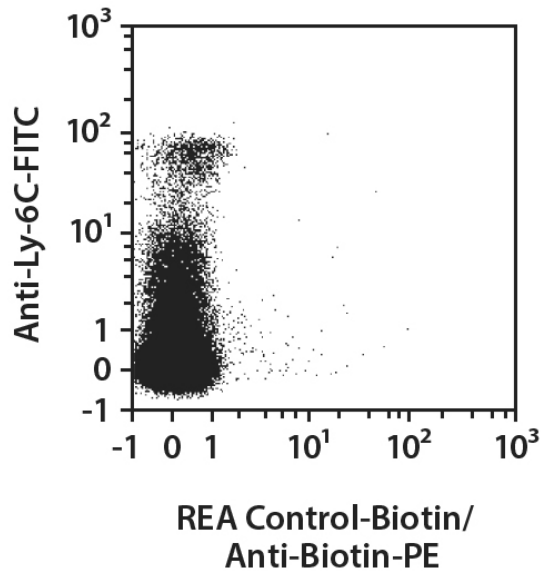

Anti-Ly-6C-PE
CD317 (PDCA-1)-PE-Vio770

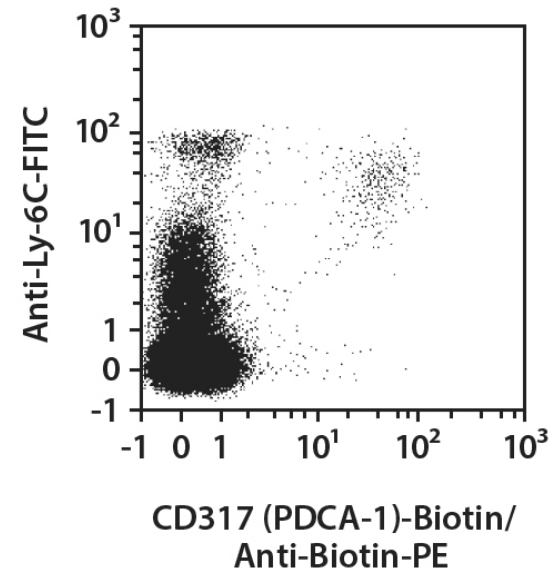












Warranty

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.