CD277 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.
CD277-PE	for 30 tests	130-108-248
CD277-PE	for 100 tests	130-108-221
CD277-APC	for 30 tests	130-108-249
CD277-APC	for 100 tests	130-108-222
CD277-Biotin	for 30 tests	130-108-247
CD277-Biotin	for 100 tests	130-108-220

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	CD277
Clone	BT3.1
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	BTN3A1, BTF5, , BTN3.1, BTN3A2, BT3.2, BTF4, BTN3.2, BT3.1
Molecular mass of antigen [kDa]	54
Distribution of antigen	B cells, cancer stem cells, dendritic cells, endothelial cells, monocytes, stromal cells, T 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 BT3.1 recognizes the human CD277 antigen, a single-pass type I membrane protein, which is also known as butyrophilin subfamily 3 member A1 (BTN3A1). CD277 belongs to the B7 family members and is expressed in various immune cells such as T cells, B cells, monocytes, NK cells, dendritic cells, a subset of stem cells, and endothelial cells. It is also consistently expressed in stromal and some tumor cells. CD277 triggering considerably enhances T cell receptor (TCR) induced cytokine production and cell proliferation. It mediates the response of T cells toward infected and transformed cells that are characterized by high levels of phosphorylated metabolites, such as isopentenyl pyrophosphate.

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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (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.

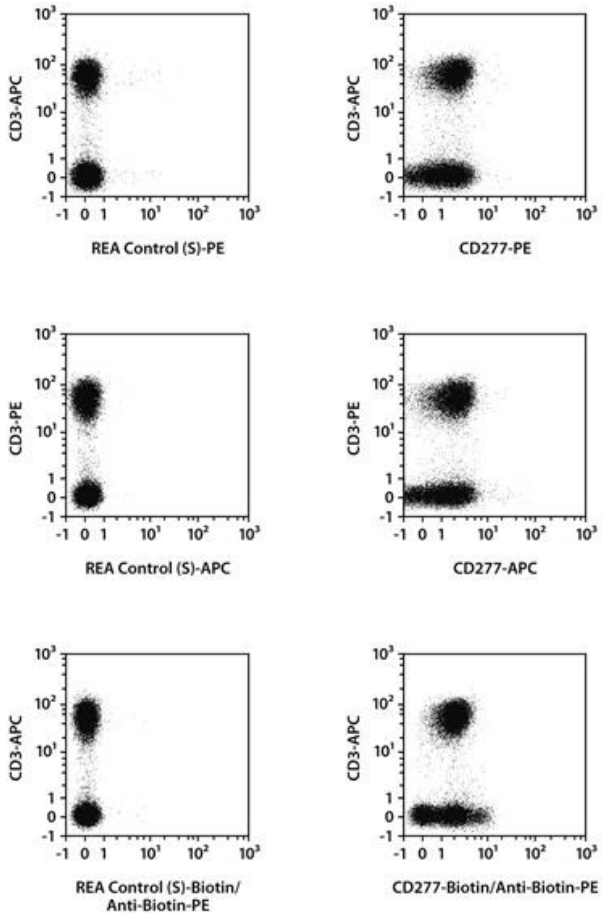
Protocol for cell surface staining

- 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 CD277 antibodies or with the corresponding isotype control antibodies (left image) as well as with CD3 antibodies. $CD14^-$ cells were pregated for the analysis. 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. **Tazi-Ahnini, R. *et al.*** (1997) Cloning, localization, and structure of new members of the butyrophilin gene family in the juxta-telomeric region of the major histocompatibility complex. Immunogenetics 47(1): 55–63.
2. **Messal, N. *et al.*** (2011) Differential role for CD277 as a co-regulator of the immune signal in T and NK cells. Eur. J. Immunol. 41(12): 3443–3454.

Warranty

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