

CD40 antibodies, human

For research use only

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

Product	Content	Order no.
CD40-PE	for 30 tests	130-099-385
CD40-PE	for 100 tests	130-094-135
CD40-APC	for 30 tests	130-099-186
CD40-APC	for 100 tests	130-094-137
CD40-Biotin	for 100 tests	130-094-142
CD40-PE-Vio770	for 30 tests	130-104-150
CD40-PE-Vio770	for 100 tests	130-104-112

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	CD40
Clone	HB14
Isotype	mouse IgG1 κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	Bp50, CDW40, TNFRSF5, p50
Molecular mass of antigen [kDa]	28
Cross-reactivity	rhesus monkey (<i>Macaca mulatta</i>), cynomolgus monkey (<i>Macaca fascicularis</i>)
Distribution of antigen	B cells, dendritic cells, endothelial cells, epithelial cells, fibroblasts, Langerhans cells, leukemia cells, lymphocytes, macrophages, mast cells, monocytes, plasma cells, platelets
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

The HB14 monoclonal antibody reacts with human CD40, a 45–50 kDa type I transmembrane glycoprotein and member of the tumor necrosis factor receptor (TNFR) superfamily. It is expressed primarily on B cells, macrophages, dendritic cells, endothelial cells, and fibroblasts and at lower levels on plasma cells and a subset of peripheral T cells. CD40 is involved in B cell differentiation and proliferation, isotype class-switching, and protection of B cells from apoptosis. Interaction of CD40 with

its ligand CD154 is important in T cell–B cell interaction and plays a role in costimulation and immune regulation.

Clone HB14 blocks the binding of CD40 to CD154.

Reagent requirements

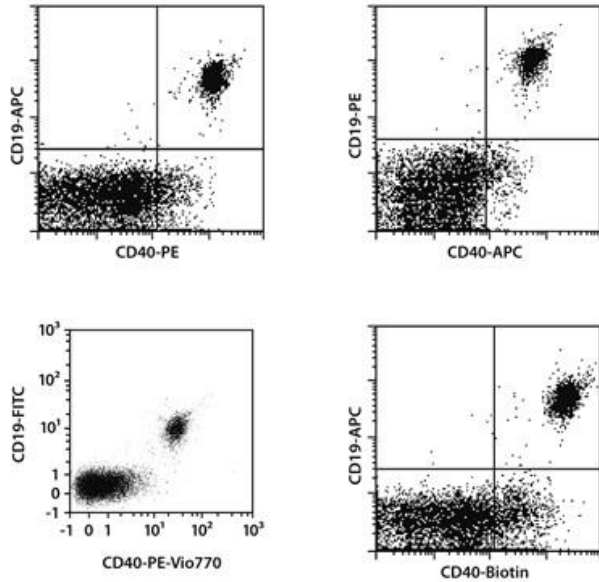
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ 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 10⁷ cells/100 µL of buffer.
 - Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ 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 10⁷ 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 CD40 antibodies as well as with CD19 antibodies and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **Kansas, G. S. et al.** (1991) Transmembrane signals generated through MHC class II, CD19, CD20, CD39, and CD40 antigens induce LFA-1-dependent and independent adhesion in human B cells through a tyrosine kinase-dependent pathway. *J. Immunol.* 147(12): 4094–4102.
2. **Banchereau, J. et al.** (1994) The CD40 antigen and its ligand. *Annu. Rev. Immunol.* 12: 881–926.

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