

CD40 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10⁶ cells.

Product	Content	Order no.
CD40-FITC	9 µg in 300 µL	130-102-887
CD40-FITC	30 µg in 1 mL	130-102-533
CD40-VioBright FITC	9 µg in 300 µL	130-105-170
CD40-VioBright FITC	30 µg in 1 mL	130-105-109
CD40-PE	9 µg in 300 µL	130-102-966
CD40-PE	30 µg in 1 mL	130-102-599
CD40-APC	30 µg in 1 mL	130-102-547
CD40-PE-Vio770	9 µg in 300 µL	130-105-417
CD40-PE-Vio770	30 µg in 1 mL	130-105-376
CD40-Biotin	9 µg in 300 µL	130-102-044
CD40-Biotin	30 µg in 1 mL	130-101-939

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	FGK45.5
Isotype	rat IgG2a
Isotype control	Rat IgG2a – isotype control antibodies
Alternative names of antigen	Bp50, gp39, HIGM1, IGM, IMD3, T-BAM, TRAP, TNFRSF5, p50
Molecular mass of antigen [kDa]	30
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	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 FGK45.5 recognizes the mouse CD40 antigen, a 40–50 kDa glycoprotein and member of the

tumor necrosis factor receptor (TNFR) family. CD40 is expressed on B lymphocytes, macrophages, and dendritic cells. It is an important costimulatory molecule for B cells as well as dendritic cells, monocytes, and other antigen-presenting cells (APCs). The interaction of CD40 on B cells with its ligand CD154 is involved in B cell activation and differentiation; engagement of CD40 on dendritic cells leads to maturation.

Clone FGK45 can be used for blocking of CD40/CD154 interaction and for *in vitro* and *in vivo* activation of CD40 expressing APCs.

Reagent requirements

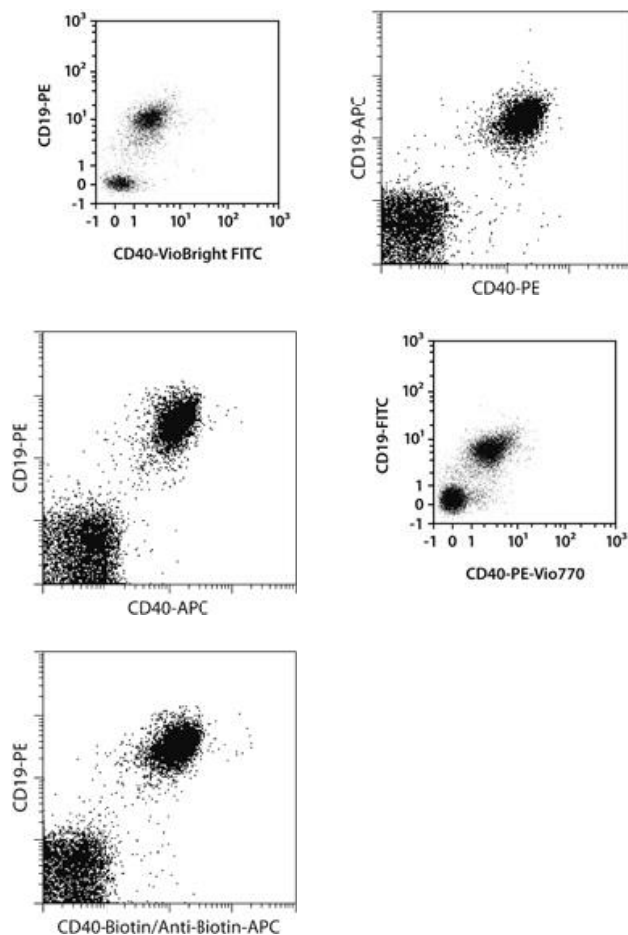
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other 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, mouse (# 130-092-575) 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 1:10 for up to 10⁶ cells/50 µL of buffer.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ 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 2×10⁶ 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 10⁶ nucleated cells per 45 µL of buffer.
 4. Add 5 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

Mouse spleen cells were stained with CD40 antibodies as well as with CD19-APC or CD19-PE and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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 either trademarks or registered trademarks of Miltenyi Biotec GmbH. Copyright © 2016 Miltenyi Biotec GmbH. All rights reserved.