CD64 (FcγRI) antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD64 (FcγRI)-PE	9 µg in 300 µL	130-103-878
CD64 (FcγRI)-PE	30 µg in 1 mL	130-103-808
CD64 (FcγRI)-APC	9 µg in 300 µL	130-103-879
CD64 (FcγRI)-APC	30 µg in 1 mL	130-103-809
CD64 (FcγRI)-PE-Vio770	9 µg in 300 µL	130-103-880
CD64 (FcγRI)-PE-Vio770	30 µg in 1 mL	130-103-810
CD64 (FcγRI)-APC-Vio770	9 µg in 300 µL	130-103-881
CD64 (FcγRI)-APC-Vio770	30 µg in 1 mL	130-103-811

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	CD64 (FcγRI)
Clone	REA286
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	FCGR1, FcgammaRI, IGGHAFC, FcR I, FcγRI
Molecular mass of antigen [kDa]	42
Distribution of antigen	dendritic cells, macrophages, monocytes, mast cells
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 REA286 recognizes the CD64 antigen, a 72 kDa integral membrane glycoprotein also known as high affinity immunoglobulin gamma Fc receptor I (FcγRI). Structurally CD64 is composed of a signal peptide that allows its transport to the surface of a cell, three extracellular immunoglobulin domains of the C2-type that it uses to bind antibody, a hydrophobic transmembrane domain, and a short cytoplasmic tail. CD64 is constitutively expressed on macrophages, monocytes, dendritic cells and mast cells. Treatment with cytokines like IFNγ and G-CSF induces CD64 upregulation. CD64 binds monomeric IgG-type antibodies with high affinity and mediates endocytosis, phagocytosis, antibody-

dependent cellular toxicity, cytokine release and superoxide generation.
Additional information: Clone REA286 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.

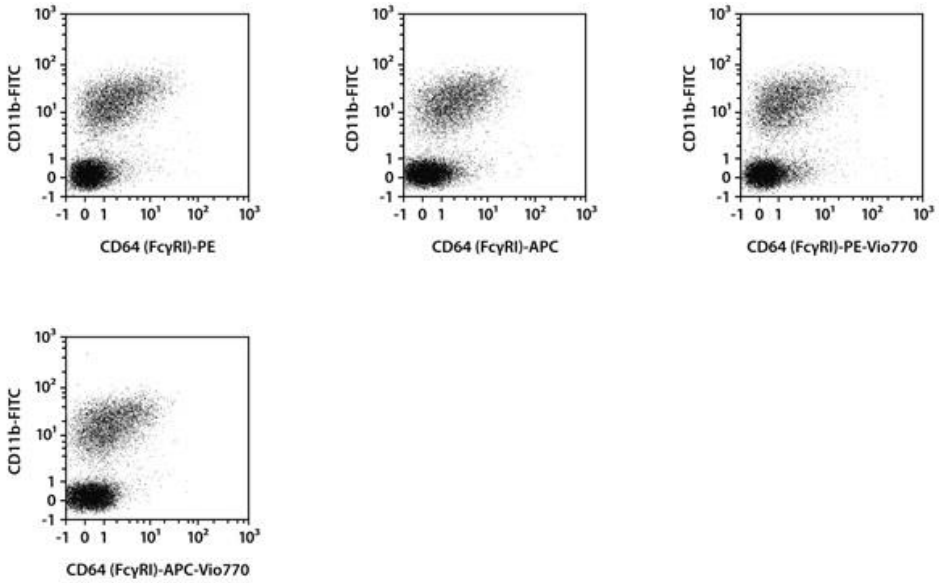
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- 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 (e.g. for 2×10^6 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 10^6 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

Bone marrow cells from C57BL/6 mice were stained with CD64 (FcγRI) antibodies, as well as with CD11b antibodies and analyzed by flow cytometry 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.



References

1. **Tan, P. S. *et al.*** (2003) Unique monoclonal antibodies define expression of FcgRI on macrophages and mast cell lines and demonstrate heterogeneity among subcutaneous and other dendritic cells. J. Immunol. 170: 2549–2556.
2. **Ioan-Facsinay, A. *et al.*** (2002) FcgammaRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. Immunity 16(3): 391–402.
3. **Nimmerjahn, F. *et al.*** (2006) Fcgamma receptors: old friends and new family members. Immunity 24(1): 19–28.

Warranty

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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**
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