CD64 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.
CD64-FITC	for 100 tests	130-097-069
CD64-PE	for 30 tests	130-100-415
CD64-PE	for 100 tests	130-097-071
CD64-APC	for 30 tests	130-100-417
CD64-APC	for 100 tests	130-097-072
CD64-VioBlue	for 30 tests	130-099-495
CD64-VioBlue	for 100 tests	130-099-488
CD64-VioGreen	for 30 tests	130-108-373
CD64-VioGreen	for 100 tests	130-108-342
CD64-PE-Vio770	for 30 tests	130-100-411
CD64-PE-Vio770	for 100 tests	130-100-413
CD64-APC-Vio770	for 30 tests	130-100-447
CD64-APC-Vio770	for 100 tests	130-100-449
CD64-PerCP-Vio700	for 30 tests	130-101-419
CD64-PerCP-Vio700	for 100 tests	130-101-422
CD64-Biotin	for 100 tests	130-097-065

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
Clone	10.1.1
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	FCGR1A, CD64A, FcR I, IGFR1, FcγRI
Molecular mass of antigen [kDa]	41
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*), chimpanzee (*Pan troglodytes*), baboon, capuchin monkey, squirrel monkey (*Saimiri sciureus*)
Distribution of antigen	dendritic cells, eosinophils, macrophages, monocytes, myeloid cells, neutrophils

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.

Clone 10.1.1 reacts with human CD64, a high affinity Fc receptor for IgG. CD64 contains three Ig-like domains in its extracellular domain and binds both monomeric and aggregated IgG and contributes to antibody-dependent cellular cytotoxicity (ADCC), phagocytosis, and the clearance of immune complexes. It is constitutively expressed on macrophages, monocytes, and eosinophils and can be up-regulated on neutrophils during infection or after stimulation with IFN-γ and colony-stimulating factors G-CSF and GM-CSF.

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

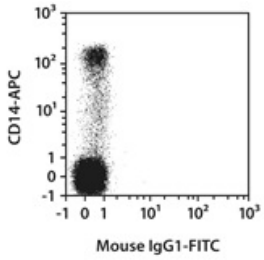
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher 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).

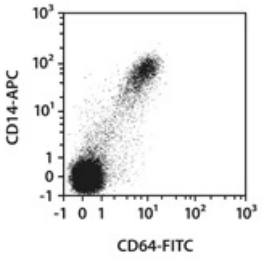
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

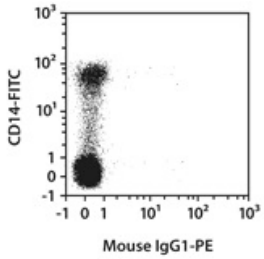
Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with CD64 antibodies or with the corresponding isotype control antibodies (left image) as well as with CD14 antibodies. Flow cytometry was performed with the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide or 4',6-diamidino-

2-phenylindole (DAPI) fluorescence, as in the case of tandem-conjugates.


CD14-APC
Mouse IgG1-FITC


CD14-APC
CD64-FITC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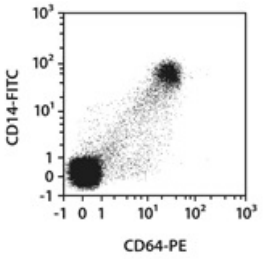
Mouse IgG1-PE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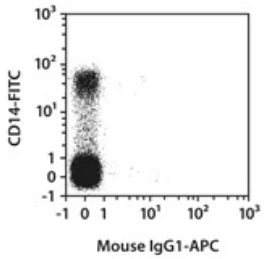
CD64-PE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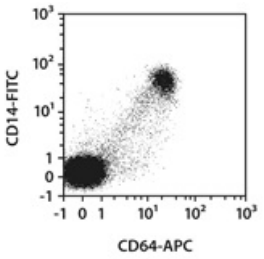
Mouse IgG1-APC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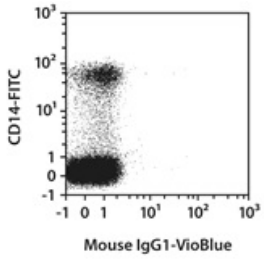
CD64-APC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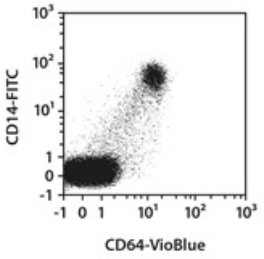
Mouse IgG1-VioBlue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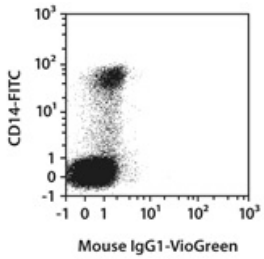
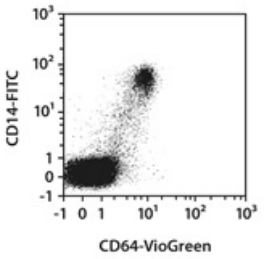
CD64-VioBlue


CD14-FITC
Mouse IgG1-VioGreen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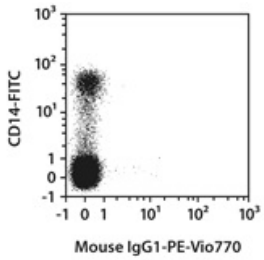
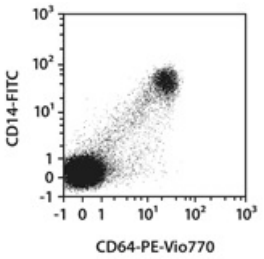
CD64-VioGreen


CD14-FITC
Mouse IgG1-PE-Vio770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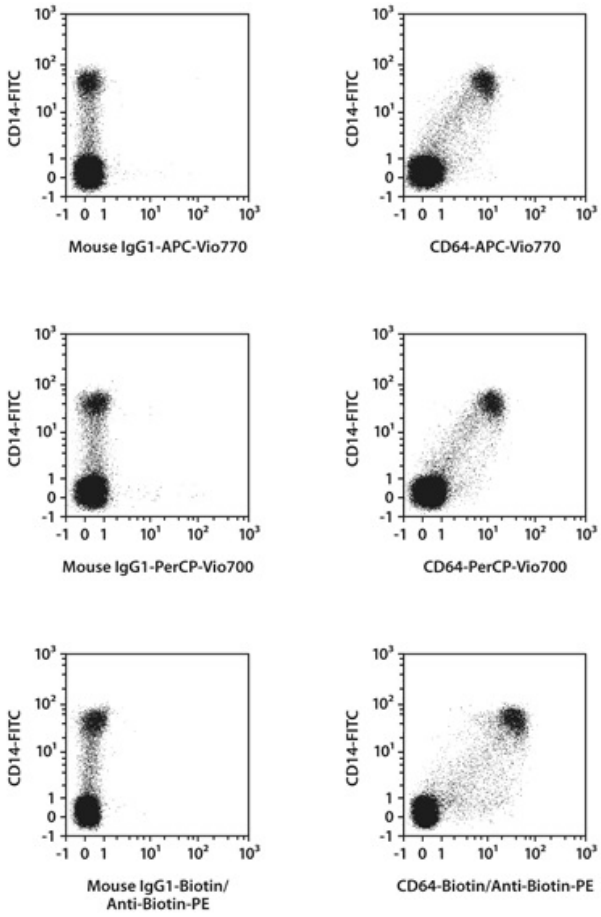
CD64-PE-Vio770



References

1. **Shen, L. *et al.*** (1987) Polymorphonuclear leukocyte function triggered through the high affinity Fc receptor for monomeric IgG. J. Immunol. 139(2): 534–538.
2. **Herra, C. *et al.*** (1996) Increased expression of Fc gamma receptors on neutrophils and monocytes may reflect ongoing bacterial infection. J. Med. Microbiol. 44(2): 135–140.
3. **Buckle, A. M. and Hogg, N.** (1989) The effect of IFN-gamma and colony-stimulating factors on the expression of neutrophil cell membrane receptors. J. Immunol. 143(7): 2295–2301.

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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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