CD14 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.
CD14-FITC	for 30 tests	130-098-063
CD14-FITC	for 100 tests	130-080-701
CD14-PE	for 30 tests	130-098-067
CD14-PE	for 100 tests	130-091-242
CD14-APC	for 30 tests	130-098-070
CD14-APC	for 100 tests	130-091-243
CD14-VioBlue	for 30 tests	130-098-058
CD14-VioBlue	for 100 tests	130-094-364
CD14-VioGreen	for 30 tests	130-098-061
CD14-VioGreen	for 100 tests	130-096-875
CD14-PerCP	for 30 tests	130-098-072
CD14-PerCP	for 100 tests	130-094-969
CD14-PE-Vio615	for 30 tests	130-107-196
CD14-PE-Vio615	for 100 tests	130-107-141
CD14-PE-Vio770	for 30 tests	130-098-074
CD14-PE-Vio770	for 100 tests	130-096-628
CD14-APC-Vio770	for 30 tests	130-098-076
CD14-APC-Vio770	for 100 tests	130-096-622
CD14-PerCP-Vio700	for 100 tests	130-097-539
CD14-Biotin	for 100 tests	130-098-380

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	CD14
Clone	TÜK4
Isotype	mouse IgG2aκ
Isotype control	Mouse IgG2a – isotype control antibodies
Alternative names of antigen	LPS R
Molecular mass of antigen [kDa]	37

Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*), pigtail monkey (*Macaca nemestrina*), cotton-top tamarin (*Saguinus oedipus*), pig, cow, sheep, goat, dog, mink, rabbit
Distribution of antigen	B cells, dendritic cells, epithelial cells, granulocytes, Langerhans cells, liver, macrophages, monocytes, osteoclasts, plasma 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 TÜK4 recognizes the human CD14 antigen and cross-reacts with non-human primate CD14. The CD14 antigen is a high affinity receptor for lipopolysaccharides (LPS) and LPS–binding protein (LBP)–complexes.[1] It is part of the functional heteromeric LPS receptor complex comprised of CD14, TLR4, and MD-2.
CD14 is strongly expressed on most human monocytes and macrophages in peripheral blood, other body fluids, and various tissues, such as lymph nodes and spleen. CD14 is expressed at high levels, also on a few CD1c (BDCA-1)$^+$ CD2$^+$ myeloid dendritic cells[2] and at low levels on neutrophilic granulocytes. *Ex vivo* differentiation of monocytes to dendritic cells is associated with down–regulation of CD14 antigen expression.

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

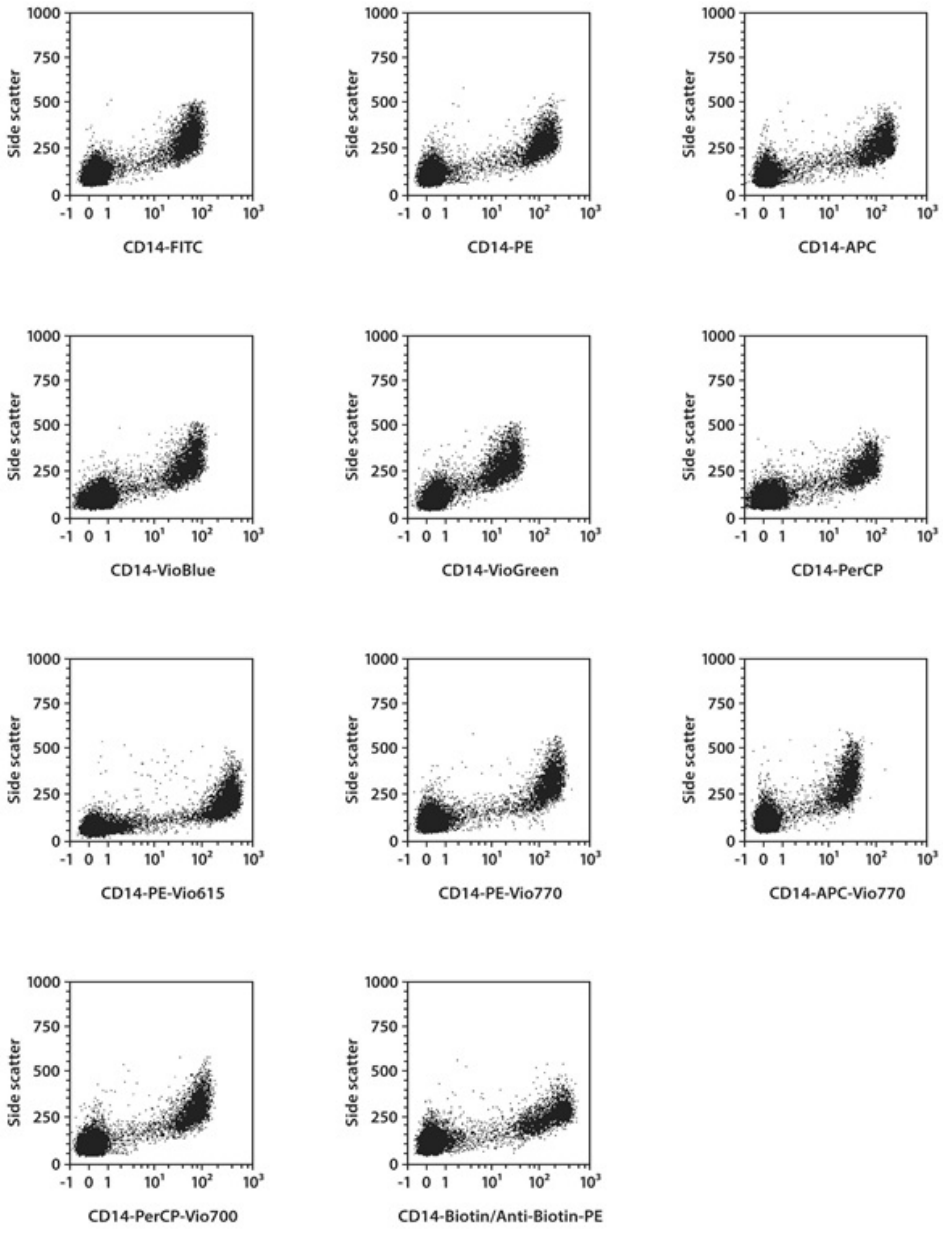
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher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

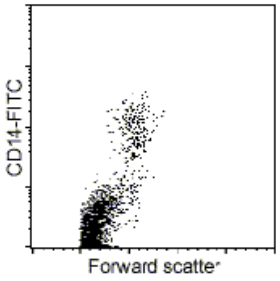
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

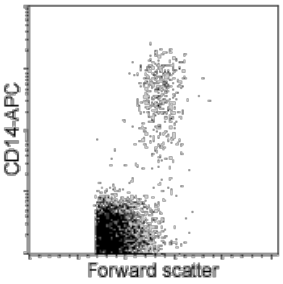
Human peripheral blood mononuclear cells (PBMCs) were stained with CD14 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



Rhesus monkey PBMCs were stained with CD14-FITC and analyzed by flow cytometry.



Cynomolgus monkey PBMCs were stained with CD14-APC and analyzed by flow cytometry.



References

1. Goyer, S. M. and Ferrero, E. (1987) Biochemical analysis of myeloid antigen and cDNA expression of gp55 (CD14). Oxford, Oxford University Press.
2. **Dzionek, A. *et al.*** (2000) BDCA-2, BDCA-3, BDCA-4: Three markers for distinct subsets of dendritic cells in human peripheral blood. J. Immunol. 165: 6037–6046.
3. **Wilson *et al.*** (1995) Selection of monoclonal antibodies for the identification of lymphocyte surface antigens in the New World primate Saguinus oedipus oedipus (cotton top tamarin). J. Immunol. Methods 178: 195–200.
4. **Jacobsen *et al.*** (1993) Reactivities of 20 anti-human monoclonal antibodies with leucocytes from ten different animal species. Vet. Immunol. Immunopathol. 39: 461–466.
5. **Meissner, F. *et al.*** (2010) Inflammasome Blood 116(9): 1570–1573.
6. **Paccou, J. *et al.*** (2013) Determination and modulation of total and surface calcium-sensing receptor expression in monocytes *in vivo* and *in vitro*. PLoS One 8(10): e74800.
7. **Neu, C. *et al.*** (2013) CD14-dependent monocyte isolation enhances phagocytosis of listeria monocytogenes by proinflammatory, GM-CSF-derived macrophages. PLoS One 8(6): e66898.

Warranty

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