Miltenyi Biotec

CD14 antibodies
human

Contents

1. Description
   1.1 Background information
   1.2 Applications
   1.3 Recommended antibody dilution
   1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD14 antibodies
4. References

1. Description

Components 1 mL monoclonal CD14 antibodies, human conjugated to various dyes.

FITC	130-080-701
PE	130-091-242
APC	130-091-243
VioBlue®	130-094-364
VioGreen™	130-096-875
PerCP	130-094-969
PE-Vio770™	130-096-628
APC-Vio770	130-096-622

Clone TÜK4 (isotype: mouse IgG2a).

Capacity 100 tests or up to 10^9 total cells.

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2−8 °C. Do not freeze. The expiration date is indicated on the vial label.

Cross-reactivity: The CD14 antibody is tested to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells. It is reported to cross-react with cotton top tamarin (*Sanguinus oedipus oedipus*) moncytes[3] and with pig, cow, sheep, goat, dog, mink, and rabbit[4].

1.1 Background information

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.

1.2 Applications

- Identification and enumeration of human monocytes/macrophages by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human monocytes can be isolated by using, for example, CD14 MicroBeads, human (# 130-050-201) or the Monocyte Isolation Kit II, human (# 130-091-153). Rhesus monkey (*Macaca mulatta*) CD14$^+$ cells can be isolated by using CD14 MicroBeads, non-human primate (# 130-091-097).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD14 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD14 MicroBead-labeled cells use the same dilution.

For optimal results, cells must be stained prior to fixation with formaldehyde.

1.4 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). 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) Mouse IgG2a isotype control antibodies conjugated to, e.g., VioBlue (# 130-094-671). For more information about isotype control antibodies refer to www. miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.

140-002-270.06

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 Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

2. General protocol for immunofluorescent staining

▲ 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).

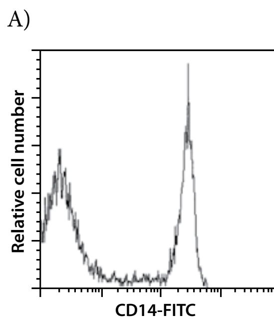
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD14 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **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. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

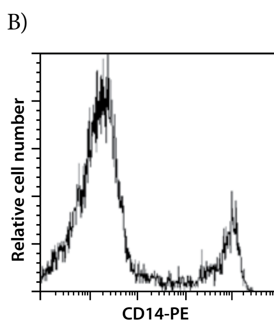
3. Examples of immunofluorescent staining with CD14 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD14 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), PerCP (F), PE-Vio770 (G), or APC-Vio770 (H) 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.

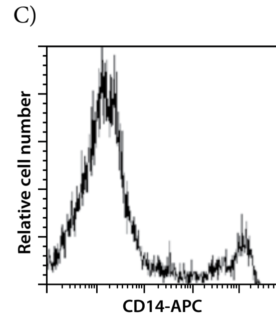
A)



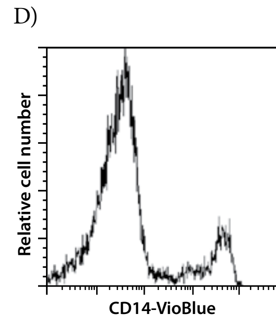
B)



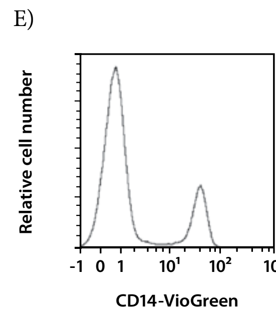
C)



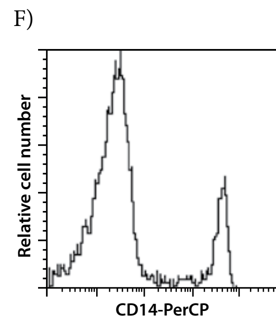
D)



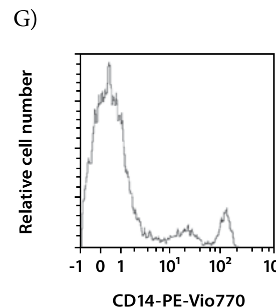
E)



F)



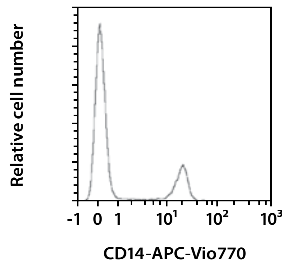
G)



140-002-270.06

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

H)



4. References

1. Goyer, S. M. and Ferrero, E. (1987) Biochemical analysis of myeloid antigen and cDNA expression of gp55 (CD14); in McMicheal, A. J. (ed.): Leucocyte Typing III White Cell Differentiation Antigens, New York, 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, A. D. *et al.* (1995) Selection of monoclonal antibodies for the identification of lymphocyte surface antigens in the New World primate *Sanguinus oedipus oedipus* (cotton top tamarin). J. Immunol. Meth. 178: 195–200.
4. Jacobsen, C. N. *et al.* (1993) Reactivities of 20 anti-human monoclonal antibodies with leukocytes from ten different animal species. Vet. Immunol. Immunopath. 39: 461–466.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

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.

autoMACS, MACS, MACSQuant, Vio, and VioBlue are registered trademarks and VioGreen and Vio770 are trademarks of Miltenyi Biotec GmbH.

Copyright © 2011 Miltenyi Biotec GmbH. All rights reserved.

140-002-270.06

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.