

CD284 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.
CD284-PE	30 µg in 1 mL	130-102-561
CD284-APC	30 µg in 1 mL	130-102-509
CD284-Biotin	30 µg in 1 mL	130-101-916

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	CD284
Clone	MTS510
Isotype	rat IgG2ak
Isotype control	Rat IgG2a – isotype control antibodies
Alternative names of antigen	TLR4, Lps, Ly87, Ran/M1, Rasl2-8
Molecular mass of antigen [kDa]	93
Distribution of antigen	dendritic cells, endothelial cells, granulocytes, leukocytes, macrophages, monocytes, placenta, spleen, T 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 MTS510 reacts with the molecular complex of CD284, a type I transmembrane signaling receptor, also known as toll-like receptor 4 (TLR4), associated with myeloid differentiation factor-2 (MD2). Toll-like receptors are a family of pattern recognition receptors detecting the presence and characteristics of an infection and induce appropriately tailored innate and adaptive immune responses. TLR4 associates with its coreceptor MD2. The resulting molecular complex is responsible for recognition and signaling upon specific lipopolysaccharide (LPS) binding. TLR4 is expressed on monocytes/macrophages as well as on myeloid dendritic cells. MTS510 detects TLR4 associated with MD2 on the surface of thioglycollate-elicited peritoneal macrophages from different mouse strains, for example, C57BL/6 or BALB/c.

Reagent requirements

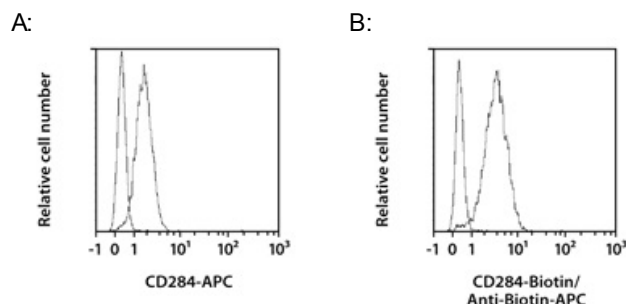
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other 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

A mixture of 90% RAW264.7 cells and 10% mouse spleen cells was stained with CD284 antibodies conjugated to APC (A) or Biotin (B), and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cells labeled with Biotin were stained with Anti-Biotin-APC. The left curves show isotype controls. 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.