CD284 antibodies
human

Contents

1. Description

Components	1 mL monoclonal CD284 antibodies, human conjugated to:
	PE 130-096-237
	APC 130-096-236
	Biotin 130-097-349
Clone	HTA125 (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 CD284 antibody has been reported to react with rabbit[6] and guinea pig[2] cells.

1.1 Background information

Clone HTA125 reacts with CD284, a type I transmembrane signaling receptor, also known as Toll-like receptor 4 (TLR4). 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 co-receptor MD-2. Together with LBP and CD14 this complex is responsible for recognition and signaling upon specific lipopolysaccharide (LPS) recognition. TLR4 is expressed on monocytes, myeloid dendritic cells, and low levels on B cells and granulocytes in human peripheral blood. HTA125 is able to block LPS induced signaling and recognizes predominantly human TLR4 associated with MD-2. [1-6]

1.2 Applications

- Identification and enumeration of $CD284^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD284 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD284-Biotin.
- (Optional) Mouse IgG2a isotype control antibodies conjugated to, e.g., PE (# 130-091-835). For more information about isotype control antibodies refer to www. miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

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2. General protocol for immunofluorescent staining

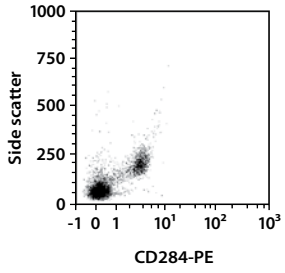
▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated 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 CD284 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 CD284-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of 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.

3. Example of immunofluorescent staining with CD284 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD284 antibodies conjugated to PE 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.



For more examples please refer to the respective product page at www.miltenyibiotec.com.

4. References

1. Shimazu, R. *et al.* (1999) MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J. Exp. Med. 189: 1777–1782.
2. Kawahara, T. *et al.* (2001) Type I *helicobacter pylori* lipopolysaccharide stimulates Toll-like receptor 4 and activates mitogen oxidase 1 in gastric pit cells. Infect. Immun. 69: 4382–4389.
3. Wang, J. *et al.* (2001) Involvement of CD14 and Toll-like receptors in activation of human monocytes by *Aspergillus fumigatus* hyphae. Infect Immun. 69: 2402–2406.
4. Wang, R. *et al.* (2003). Characterization of monoclonal antibody HTA125 with specificity for human TLR4. Hybrid Hybridomics 22: 357–365.
5. Jin, M.S. and Lee, J-O. (2008) Structures of the Toll-like receptor family and its ligand complexes. Immunity 29: 182–191.
6. Burgener, I. *et al.* (2008) Antibodies specific for human or murine Toll-like receptors detect canine leukocytes by flow cytometry. Vet. Immunol. Immunopathol. 124: 184–191.

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

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