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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.

1. Description

This product is for research use only.

Components Monoclonal CD45 antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-080-202	130-098-043
VioBright™ FITC	130-104-517	130-104-566
PE	130-080-201	130-098-141
APC	130-091-230	130-098-143
VioBlue®	130-092-880	130-098-136
VioGreen™	130-096-906	130-098-138
PerCP	130-094-975	130-098-145
PE-Vio770™	130-096-616	130-098-148
APC-Vio770™	130-096-609	130-098-151
PerCP-Vio700™	130-097-527	–
Biotin	130-098-551	130-098-550

Clone 5B1 (isotype: mouse IgG2a).

Capacity 1 mL: 100 tests or up to 10⁹ total cells
300 µL: 30 tests or up to 3×10⁸ 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.

1.1 Background information

- Antigen: CD45
- Synonym: LCA; T200
- Expression patterns: The CD45 antibody recognizes the human CD45 antigen, a tyrosine phosphatase also known as the leukocyte common antigen (LCA). The CD45 molecule is required for T and B cell activation and is expressed in at least five isoforms depending on the differentiation status of the cell. The CD45 antibody recognizes a common epitope of all CD45 isoforms.

1.2 Applications

- Identification and enumeration of CD45⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD45 conjugates is **1:11 for up to 10⁷ 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). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye-conjugated antibodies to human cells, especially to monocytes.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD45-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/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.

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).

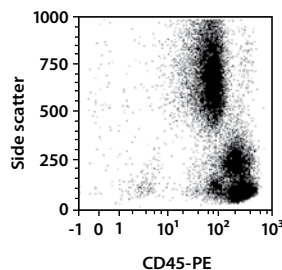
1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD45 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ\text{C}$).

▲ **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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD45-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 CD45 antibodies

Lysed human whole blood was stained with CD45 antibodies conjugated to PE. Cells were analyzed by using the MACSQuant® Analyzer.



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

Refer to www.miltenyibiotec.com for all data sheets and protocols.

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