Miltenyi Biotec

CD45RA antibodies
human

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1. Description

Components 1 mL monoclonal CD45RA antibodies, human conjugated to various dyes.

FITC	130-092-247
PE	130-092-248
APC	130-092-249
VioBlue®	130-095-464
VioGreen™	130-096-921
PerCP	130-095-466
PE-Vio770™	130-097-577
APC-Vio770	130-096-604
PerCP-Vio700	130-097-693
Biotin	130-096-259

Clone T6D11 (isotype: mouse IgG2b).

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 CD45RA antibody has been reported to react with rhesus monkey (*Macaca mulatta*) cells.

1.1 Background information

CD45RA is expressed on $CD4^+$ and $CD8^+$ T cell subsets, as well as on subsets of B cells, NK cells, and monocytes. The CD45RA antibody recognizes the 220 kDa isoform of the leukocyte common antigen (LCA), a transmembrane tyrosine phosphatase.

1.2 Applications

- Identification and enumeration of $CD45RA^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human lymphocytes can be isolated by using, for example, CD45RA MicroBeads, human (# 130-045-901).
- Studies of thymocyte differentiation and T cell activation.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD45RA conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD45RA MicroBead-labeled cells use the same dilution.

The antibody is suited for staining of formaldehyde-fixed cells.

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 CD45RA-Biotin.
- (Optional) CD45RO-FITC (# 130-095-462) or CD45RO-PE (# 130-095-457). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG2b isotype control antibodies conjugated to, e.g., PE (# 130-092-215). 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.

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 CD45RA antibody.

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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. (Optional) If CD45RA-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.

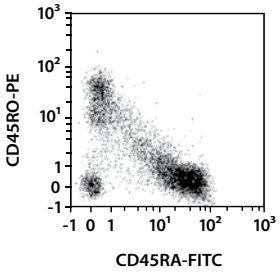
7. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

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

3. Examples of immunofluorescent staining with CD45RA antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD45RA antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), PerCP (F), PE-Vio770 (G), APC-Vio770 (H), or PerCP-Vio700 (I) as well as with CD45RO-FITC (# 130-095-462) or CD45RO-PE (# 130-095-457) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD45RA-Biotin (J) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD45RO-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A)



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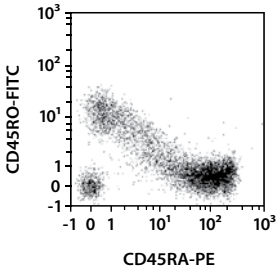

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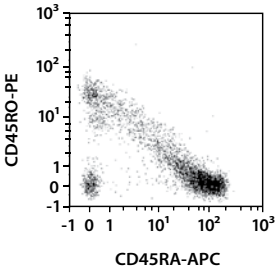

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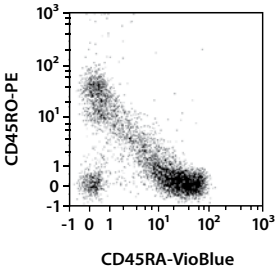

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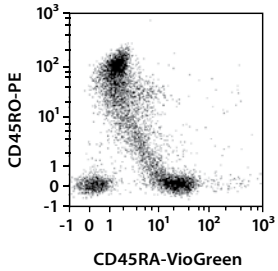

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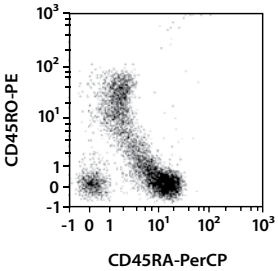
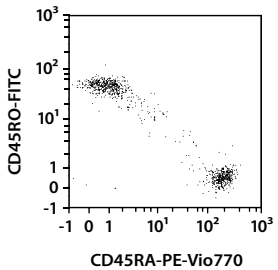


G)



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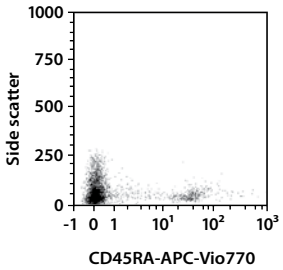
H)



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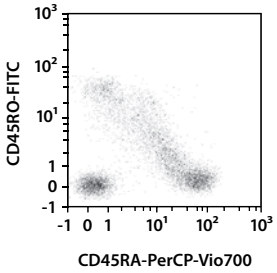

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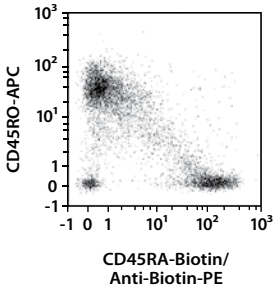

All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

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