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### 1. Description

<b>Components</b>	1 mL monoclonal CD45 antibodies, human conjugated to various dyes.	
	FITC	130-080-202
	PE	130-080-201
	APC	130-091-230
	VioBlue®	130-092-880
	VioGreen™	130-096-906
	PerCP	130-094-975
	PE-Vio770™	130-096-616
	APC-Vio770	130-096-609
<b>Clone</b>	5B1 (isotype: mouse IgG2a).	
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.	
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.	
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.	

#### 1.1 Background information

The CD45 antigen is a tyrosine phosphatase, also known as leukocyte common antigen (LCA). CD45 is present on all human cells of hematopoietic origin, except erythroid cells, platelets and their precursor cells. The CD45 molecule is required for T cell and B cell activation and is expressed in at least 5 isoforms, depending on the activation status of the cell.

#### 1.2 Applications

- Identification and enumeration of human leukocytes by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human leukocytes can be isolated by using CD45 MicroBeads, human (# 130-045-801) and Whole Blood CD45 MicroBeads (# 130-090-872).

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD45 conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD45 MicroBead-labeled cells use the same dilution.

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<sup>2+</sup> or Mg<sup>2+</sup> 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., PE (# 130-091-835). For more information about isotype control antibodies refer to [www.miltenyibiotec.com](http://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<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> 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<sup>7</sup> 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 °C).

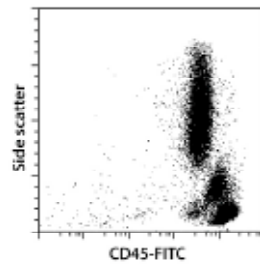
▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

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.

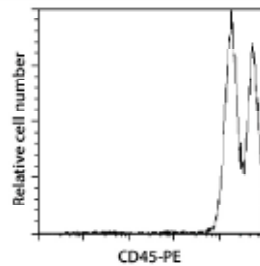
### 3. Examples of immunofluorescent staining with CD45 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD45 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. Graphs (E), (G), and (H) show gating on lymphocytes only. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

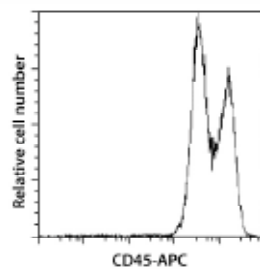
A)



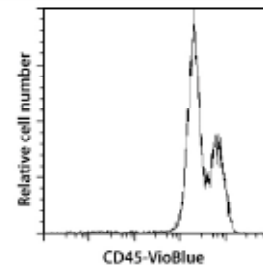
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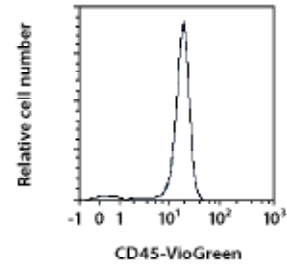
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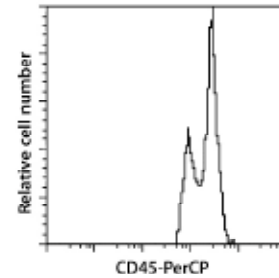
D)



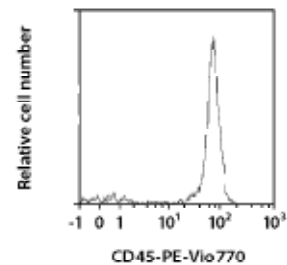
E)



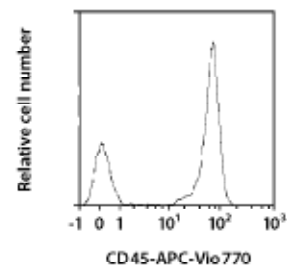
F)



G)



H)



**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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