CD45 antibodies
non-human primate

CD45-FITC	130-091-898
CD45-PE	130-091-897
CD45-APC	130-091-900
CD45-Biotin	130-091-901

Antibodies

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1. Description

Clone MB4-6D6 (isotype: mouse IgG1).

Product format 1 mL CD45 antibodies, non-human primate: monoclonal CD45 antibodies conjugated to fluorescein-isothiocyanate (**FITC**), R-phycoerythrin (**PE**), allophycocyanin (**APC**), or biotin (**Biotin**).
The antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size For 10^9 total cells, up to 100 stainings.

Storage Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The monoclonal CD45 antibody recognizes non-human primate leukocytes. The antibody reacts similar to human CD45 antibodies binding to the human leukocyte common antigen (LCA). CD45 is expressed on non-human primate lymphocytes, monocytes and granulocytes. It is not expressed on erythroid cells.
The CD45 antibody recognizes the CD45 antigen of **rhesus monkey (*Macaca mulatta*)**, **cynomolgus monkey (*Macaca fascicularis*)** and **pigtail monkey (*Macaca nemestrina*)** cells. Cross-reactivity with other non-human primates has not been tested. The antibody also recognizes the human CD45 antigen.

Product applications

- Identification and enumeration of $CD45^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy. Rhesus monkey (*Macaca mulatta*) leukocytes can be isolated by using CD45 MicroBeads, non-human primate (# 130-091-899).
- Evaluation of MACS separations by flow cytometry or fluorescence microscopy by staining the antigen of interest and counterstaining of leukocytes with CD45 antibodies.

1.2 Examples of staining concentrations
for non-human primate cells.

CD45-conjugate	FITC	PE	APC	Biotin
	Recommended antibody dilution[a]			
Flow cytometry				
- in general	1:11	1:11	1:11	1:11
- formaldehyde-fixed cells	1:11	1:11	1:11	1:11
- CD45 MicroBead-labeled cells	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to $1x10^8$ cells/mL buffer.

- The CD45 antibody cross-reacts with human cells.

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA, e.g. by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) For flow cytometric exclusion of dead cells without cell fixation, PI (propidium iodide) or 7-AAD. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163). For fluorescent staining of CD45-Biotin, Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856).

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10^7 total 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 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend 10^7 cells in 100 µL of buffer.
2. Add 10 µL of CD45 antibodies.
3. Mix well and incubate for 10 minutes in the dark at 4–8 °C.

   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.

   If CD45-Biotin was used, resuspend the cell pellet in 100 µL buffer, add 10 µL Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC, and continue as described in step 3 and 4.
5. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

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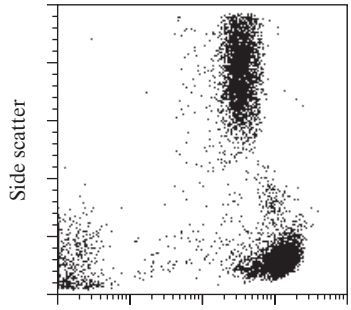
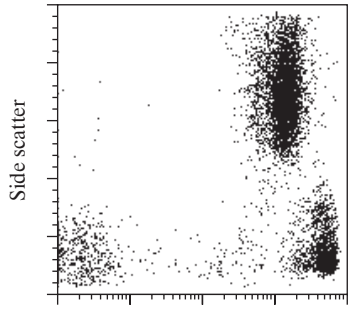
3. Examples of immunofluorescent staining with CD45 antibodies

Rhesus monkey peripheral blood leukocytes (PBL) were stained with CD45 antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

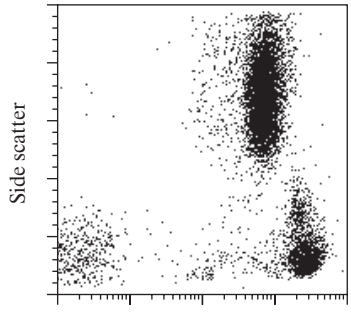
(a) Rhesus monkey PBL stained with CD45-FITC.



(b) Rhesus monkey PBL stained with CD45-PE.

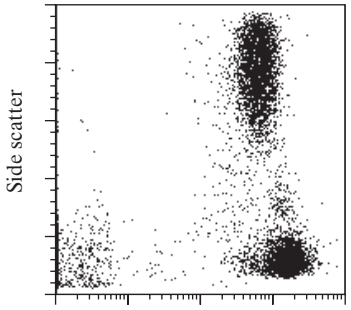


(c) Rhesus monkey PBL stained with CD45-APC.



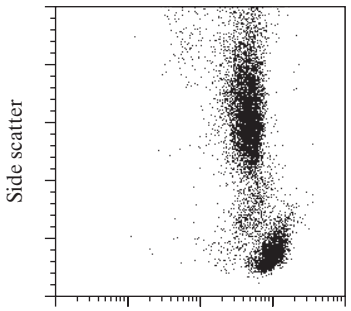
Rhesus monkey PBL (d) were labeled with CD45-Biotin and Anti-Biotin-APC and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(d) Rhesus monkey cells stained with CD45-Biotin and Anti-Biotin-APC.



Cynomolgus monkey PBL (e) were labeled with CD45-FITC and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(e) Cynomolgus monkey cells stained with CD45-FITC.



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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