



Antibodies

Index

1. Description

1.1 Background and product applications

1.2 Examples of staining concentrations

1.3 Reagent requirements

2. General protocol for immunofluorescent staining

3. Examples of immunofluorescent staining with CD16 antibodies

1. Description

Clone	VEP13 (isotype: mouse IgM).
Product format	1 mL CD16 antibodies: monoclonal CD16 antibodies conjugated to fluorescein-isothiocyanate (FITC), R-phycoerythrin (PE), or allophycocyanin (APC). 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 CD16 antigen is a 50–80 kDa glycoprotein that is expressed in two different isoforms. The transmembrane form is found on NK cells, macrophages and mast cells. The glycosylphosphatidylinositol (GPI)-linked form is present on neutrophils. The CD16 antigen is a low affinity receptor for aggregated IgG. The transmembrane form plays a role in signal transduction, NK cell activation and antibody-dependent cellular cytotoxicity. This fluorochrome-conjugated antibody recognizes both the extracellular domain of the transmembrane form as well as the GPI-linked form of the human CD16 antigen.

The CD16 antibodies also recognize a subset of rhesus monkey (*Macaca mulatta*) lymphocytes. CD16 is expressed on rhesus monkey NK cells and a subset of monocytes, but not on granulocytes.

Product applications

- Identification and enumeration of CD16⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS[®] separations by flow cytometry or fluorescence microscopy. Human CD16⁺ cells can be isolated by using, e.g. CD16 MicroBeads, human (# 130-045-701). Rhesus monkey (*Macaca mulatta*) CD16⁺ cells can be isolated by using CD16 MicroBeads, non-human primate (# 130-091-145).

CD16 antibodies

human

CD16-FITC	130-091-244
CD16-PE	130-091-245
CD16-APC	130-091-246

1.2 Examples of staining concentrations

for human and non-human primate cells.

CD16-conjugate	FITC	PE	APC
Recommended antibody dilution ^a			
Flow cytometry			
- in general	1:11	1:11	1:11
- formaldehyde-fixed cells	1:11 ^b	1:11 ^b	1:11 ^b
- CD16 MicroBead-labeled cells	1:11	1:11	1:11
a) Given antibody dilutions are for a cell concentration of up to 1×10 ⁸ cells/mL buffer.			
b) For optimal results, human and non-human primate cells have to be stained prior to fixation.			

- The CD16 antibody is reported to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA 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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10⁷ total cells. When working with fewer than 10⁷ 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 2×10⁷ total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend 10⁷ cells in 100 µL of buffer.
2. Add 10 µL of CD16 antibodies.
▲ **Note:** See table for exceptions.
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 10⁷ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
5. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

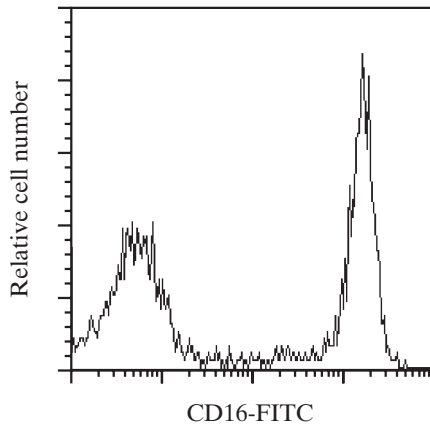


3. Examples of immunofluorescent staining with CD16 antibodies

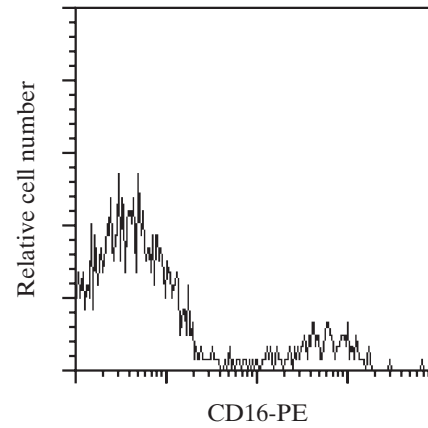
Human lysed blood was stained with CD16 antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

Rhesus monkey PBMCs (d) were stained with CD16-PE and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

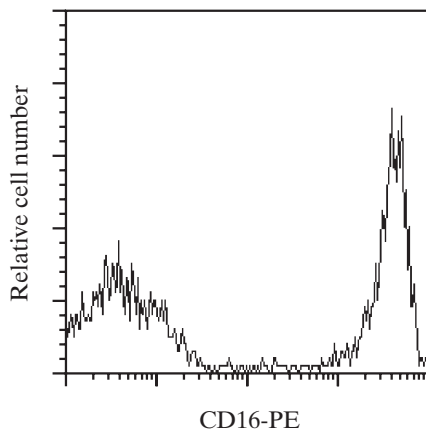
(a) Human lysed blood stained with CD16-FITC.



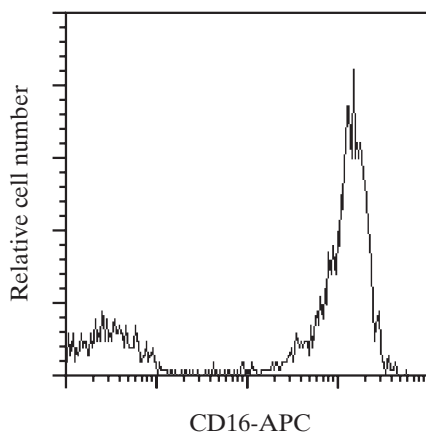
(d) Rhesus monkey PBMCs stained with CD16-PE.



(b) Human lysed blood stained with CD16-PE.



(c) Human lysed blood stained with CD16-APC.



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.

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