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1. Description

Components	1 mL monoclonal CD16 antibodies, human conjugated to various dyes.	
	FITC	130-091-244
	PE	130-091-245
	APC	130-091-246
	VioGreen™	130-096-879
	APC-Vio770™	130-096-655
Clone	VEP13 (isotype: mouse IgM).	
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 CD16 antibody has been reported to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

1.1 Background information

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.

1.2 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).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD16 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD16 MicroBead-labeled cells use the same dilution.

The antibody is suited for staining of formaldehyde-fixed cells. For optimal results, human and non-human primate cells must be stained prior to fixation with formaldehyde.

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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Mouse IgM isotype control antibodies conjugated to, e.g., PE (# 130-093-177). 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

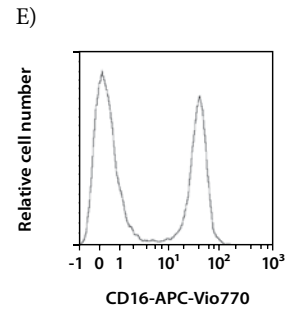
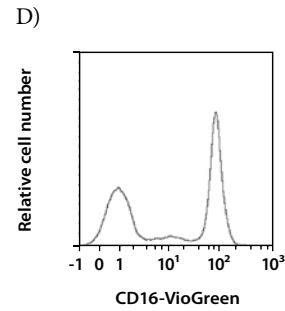
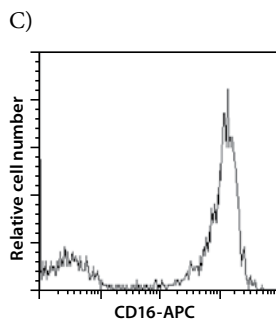
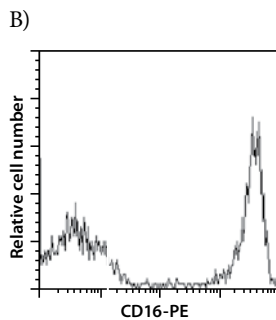
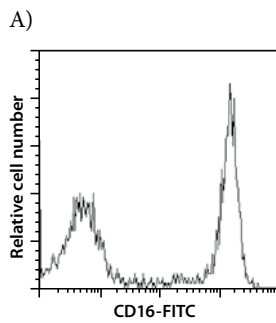
▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 10⁷ nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD16 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

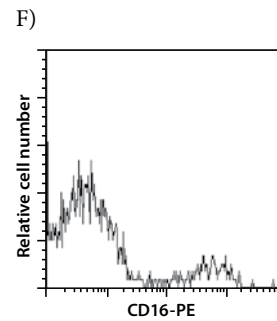
▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD16 antibodies

Human lysed blood was stained with CD16 antibodies conjugated to FITC (A), PE (B), APC (C), VioGreen (D), or APC-Vio770 (E) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



Rhesus monkey peripheral blood mononuclear cells (PBMCs) (F) 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 propidium iodide fluorescence.



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.

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