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1. Description

Components	1 mL monoclonal CD15 antibodies, human conjugated to various dyes.	
	FITC	130-081-101
	PE	130-091-375
	APC	130-091-371
	VioGreen™	130-096-877
Clone	VIMC6 (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.	

1.1 Background information

The CD15 antigen is expressed on human myelomonocytic cells. It is present on neutrophils, eosinophils, and some monocytes, but not on basophils or lymphocytes. The antigen is also expressed on Hodgkin/Reed-Sternberg cells.

The CD15 antibody recognizes the carbohydrate structure 3-fucosyl-N-acetyl-lactosamine. It specifically labels neutrophils, eosinophils, and monoblastoid precursor cells of the myeloid lineage.

1.2 Applications

- Identification and enumeration of CD15⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human eosinophils and neutrophils can be isolated by using, for example, CD15 MicroBeads (# 130-146-601).

1.3 Recommended antibody dilution

When using CD15-FITC the dilution is **1:22 for up to 10⁸ cells/mL** of buffer for labeling of cells and analyzing by flow cytometry.

For all other conjugates the dilution is **1:11 for up to 10⁸ cells/mL**. For CD15 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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Mouse IgM isotype control antibodies conjugated to, e.g., FITC (# 130-093-178). 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

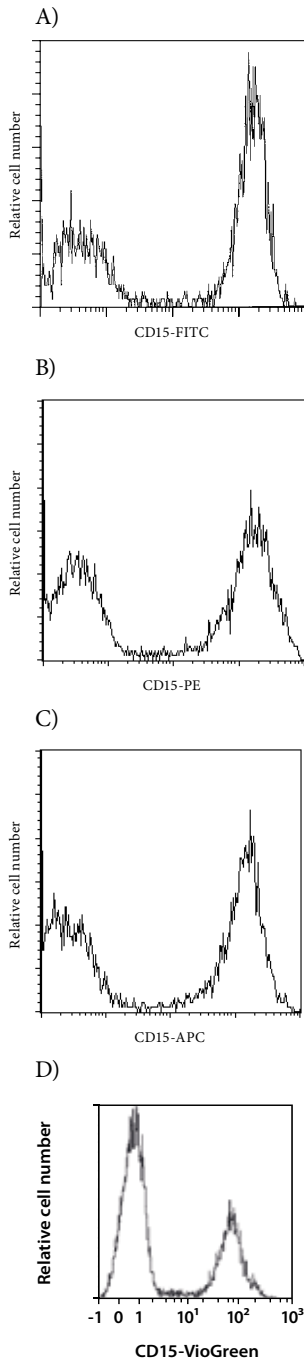
▲ Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 CD15 antibody.
 - ▲ **Note:** Refer to section 1.3 for exceptions.
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 CD15 antibodies

Human cells from lysed blood were stained with CD15 antibodies conjugated to FITC (A), PE (B), APC (C), or VioGreen (D) 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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