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1. Description

Components	1 mL monoclonal CD19 antibodies, mouse conjugated to various dyes.
	FITC 130-092-042
	PE 130-092-041
	APC 130-092-039
	PE-Vio770™ 130-097-207
	APC-Vio770 130-097-213
Clone	6D5 (isotype: rat IgG2a).
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

CD19, a 95 kDa type-I transmembrane glycoprotein, is a B cell differentiation antigen expressed on B cells throughout their development from the early pro-B cell through the mature B cell stages. Its expression is down-regulated during terminal differentiation to plasma cells. CD19 associates on the cell surface of mature B cells with complement receptor CD21 and with CD81, forming a multimolecular complex with synergistic signaling to membrane IgM. CD19 is a critical regulator of B cell development, activation and differentiation. It is also expressed on follicular dendritic cells and peritoneal mast cells.

1.2 Applications

- Identification and enumeration of CD19⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy, for example:
 - Positive selection or depletion of mouse B cells by using CD19 MicroBeads, mouse (# 130-052-201) or CD45R (B220) MicroBeads, mouse (# 130-049-501);

- Isolation of untouched resting B cells by using the B Cell Isolation Kit, mouse (# 130-090-862);
- Positive selection or depletion of mouse B-1 cells by using CD5 (Ly-1) MicroBeads, mouse (# 130-049-301).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD19 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD19 MicroBead-labeled cells use the same dilution. CD19-FITC and CD19-APC-Vio770 for staining of cells labeled with CD19 MicroBeads are not recommended.

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (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

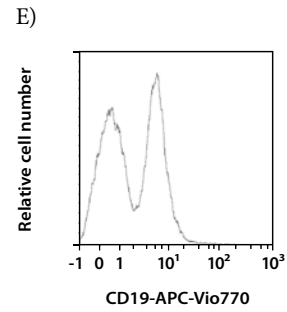
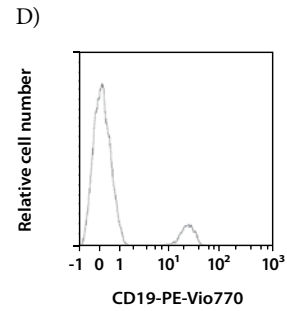
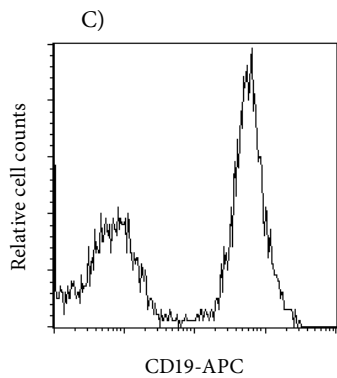
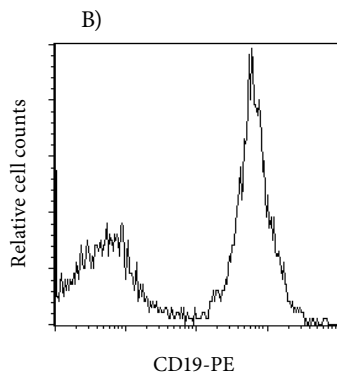
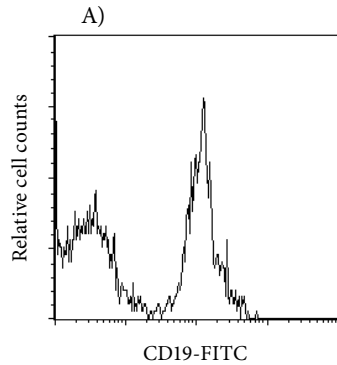
▲ Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 CD19 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 CD19 antibodies

Mouse spleen cells were stained with CD19 antibodies conjugated to FITC (A), PE (B), APC (C), PE-Vio770 (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.



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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