

### Contents

1. Description
  - 1.1 Background information
  - 1.2 Applications
  - 1.3 Recommended antibody dilution
  - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD19 antibodies
4. References

### 1. Description

<b>Components</b>	1 mL monoclonal CD19 antibodies, human conjugated to various dyes.	
	FITC	130-091-328
	PE	130-091-247
	APC	130-091-248
	PE-Vio770™	130-096-641
	APC-Vio770	130-096-643
<b>Clone</b>	LT19 (isotype: mouse IgG1).	
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.	
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.	
<b>Storage</b>	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 is a 95 kDa type-I transmembrane glycoprotein that belongs to the immunoglobulin superfamily. It is expressed on B cells throughout most stages of B cell differentiation, but the expression is down-regulated during the terminal differentiation to plasma cells. Expression of CD19 is also found in the majority of B cell-derived malignancies. CD19 is further present on follicular dendritic cells. On B cells, CD19 associates with CD21, CD81, and CD225 (Leu-13) forming a signal transduction complex. CD19 is a critical regulator in B cell development, activation, and differentiation.<sup>1-3</sup>

#### 1.2 Applications

- Identification and enumeration of human B cells by flow cytometry or fluorescence microscopy.
- Identification and enumeration of follicular dendritic cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human B cells can be isolated by using, for example, CD19 MicroBeads (# 130-050-301), the CD19 MultiSort Kit (# 130-055-301), CD20 MicroBeads

(# 130-091-104), CD22 MicroBeads (# 130-046-401), or the B Cell Isolation Kit II (# 130-091-151). Human memory B cells (CD19<sup>+</sup>CD27<sup>+</sup>) can be isolated by combining CD27 MicroBeads (# 130-051-601) with the CD19 MultiSort Kit or the B Cell Isolation Kit II. Human naive B cells (CD19<sup>+</sup>IgD<sup>+</sup>) can be isolated by using the Naive B Cell Isolation Kit II (# 130-091-150). Human resting B cells (CD19<sup>+</sup>CD43<sup>-</sup>) can be isolated by using CD43 MicroBeads (# 130-091-333).

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD19 conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD19 MicroBead-labeled cells use the same dilution. To use CD19-FITC for staining of cells labeled with CD19 MicroBeads is not recommended.

The antibody is suited for staining of formaldehyd-fixed cells.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

#### 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD19 MicroBeads, human (# 130-050-301).
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212). For more information about isotype control antibodies refer to [www.miltenyibiotec.com](http://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.

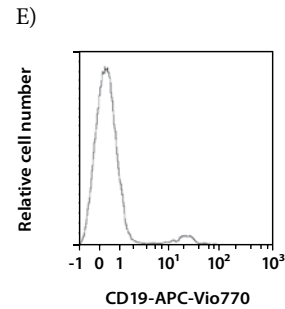
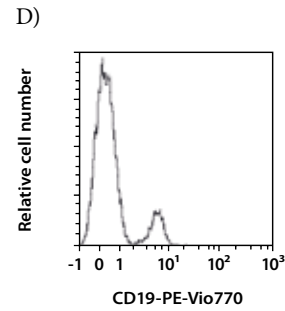
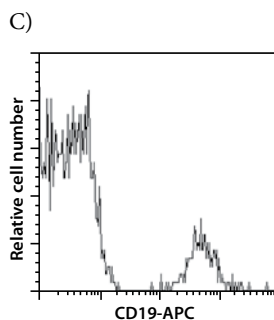
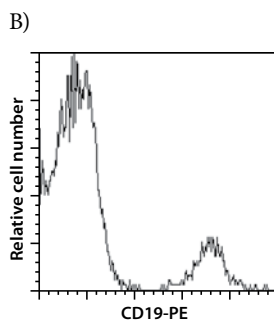
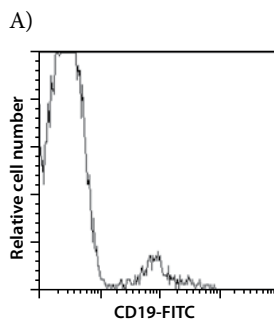
### 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> 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 10<sup>7</sup> 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

Human peripheral blood mononuclear cells (PBMCs) 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 using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



### 4. References

1. Nadler, L. M. *et al.* (1983) B4, a human B lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. *J. Immunol.* 131: 244–250.
2. Tedder, T. F. *et al.* (2002) CD19-CD21 complex regulates an intrinsic Src family kinase amplification loop that links innate immunity with B-lymphocyte intracellular calcium responses. *Biochem. Soc. Trans.* 30: 807–811.
3. Poe, J. C. *et al.* (2001) CD19, CD21, and CD22: multifaceted response regulators of B lymphocyte signal transduction. *Int. Rev. Immunol.* 20: 739–762.
4. Fujimoto, M. *et al.* (2000) CD19 regulates intrinsic B lymphocyte signal transduction and activation through a novel mechanism of processive amplification. *Immunol. Res.* 22: 281–298.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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.

#### Warranty

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