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1. Description

Components	1 mL monoclonal CD20 antibodies, human conjugated to various dyes.
	FITC 130-091-108
	PE 130-091-109
	APC 130-097-619
	VioBlue® 130-094-167
	VioGreen™ 130-096-904
	PerCP 130-094-976
Clone	LT20 (isotype: mouse IgG1).
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 CD20 antibody has been tested to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

1.1 Background information

The CD20 antigen is a 33–37 kDa non-glycosylated transmembrane protein that is phosphorylated upon activation. CD20 is suggested to function as a specialized, differentiation-associated calcium channel enabling high local calcium concentrations necessary for signaling events¹.

The CD20 antigen is expressed exclusively on B cells (pre-B cells, naive and memory B cells), but not on early B cell progenitors or plasma cells. It also has been found on the majority of B cell lineage malignancies and has been used as a target antigen for antibody-based immunotherapy of B cell lymphomas².

This monoclonal antibody reacts with a short extracellular domain of the CD20 antigen. In contrast to other antibodies, including the ones used for immunotherapy, clone LT20 induces neither apoptosis nor complement lysis³.

1.2 Applications

- Identification and enumeration of human B cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human CD20⁺ can be isolated by using, for example, CD20 MicroBeads (# 130-091-104), CD19 MicroBeads (# 130-050-301), CD19 MultiSort Kit (# 130-055-301), CD22 MicroBeads (# 130-046-401), or the B Cell Isolation Kit II (# 130-091-151).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD20 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD20 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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (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.
- (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).

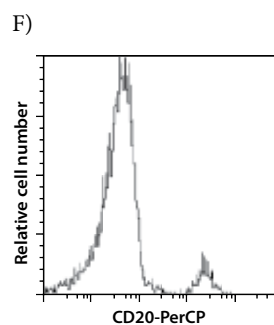
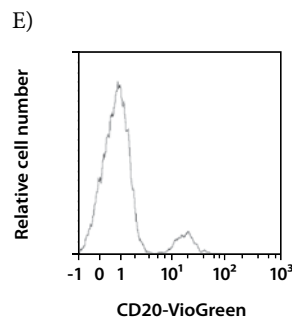
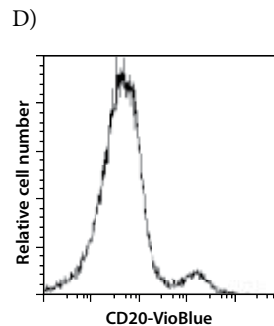
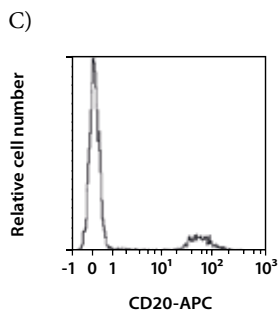
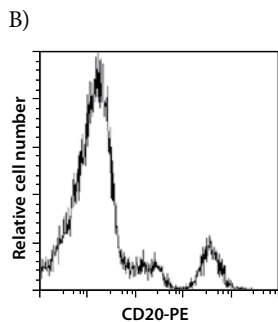
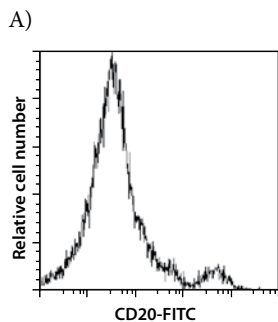
1. Determine cell number.

2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add 20 µL FcR Blocking Reagent to 10⁷ total cells resuspended in 80 µL of buffer.
4. Add 10 µL of the CD20 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 CD20 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD20 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), or PerCP (F) 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. Polyak, M. J. and Deans, J. P. (2002) CD20 Workshop Panel report; in Mason, D. *et al.* (eds.): Leucocyte typing VII, Oxford, Oxford University Press.
2. Countouriotis, A. *et al.* (2002) Cell surface antigen and molecular targeting in the treatment of hematologic malignancies. Review. *Stem cells* 20: 215–229.
3. Cragg, M. S. *et al.* (2002) Opposing properties of CD20 mAb; in: Mason, D. *et al.* (eds.): Leucocyte typing VII, Oxford, Oxford University Press.

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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