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### 1. Description

<b>Components</b>	1 mL monoclonal CD21/CD35 antibodies, mouse conjugated to:
	FITC 130-096-386
	PE 130-096-411
	APC 130-096-413
	PE-Vio770™ 130-097-216
	Biotin 130-096-384
<b>Clone</b>	7E9 (isotype: rat IgG2a).
<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

Clone 7E9 reacts with an epitope shared by mouse CD21 and CD35 also known as CR2 and CR1. CD21 and CD35 are alternatively spliced transcripts from the Cr2 gene, which produce cell-surface proteins of 145 and 190 kDa, respectively.

CD21 and CD35 are expressed on the majority of peripheral B lymphocytes, on the majority of resident peritoneal macrophages, on mast cells, activated granulocytes and follicular dendritic cells. CD21 forms part of the signal-transduction complex with CD19 and CD81 which are associated with the antigen receptor on B lymphocytes.

#### 1.2 Applications

- Identification and enumeration of CD21/CD35<sup>+</sup> cells by flow cytometry.

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD21/CD35 conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

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 Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD21/CD35-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

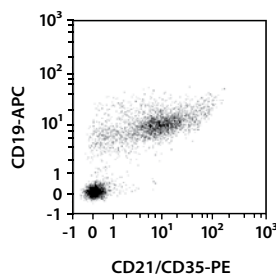
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^7$  nucleated cells. When working with fewer than  $10^7$  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  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the CD21/CD35 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **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  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD21/CD35-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  of anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

## 3. Example of immunofluorescent staining with CD21/CD35 antibodies

Mouse spleen cells (BALB/c) were stained with CD21/CD35 antibodies conjugated to PE as well as with CD19-APC (# 130-092-039) 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.



For more examples please refer to the respective product page at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

## 4. References

1. Konzono, Y. *et al.* (1998) Cross-linking CD21/CD35 or CD19 increases both B7-1 and B7-2 expression on murine splenic B cells. *J. Immunol.* 160: 1565–1572.
2. Oliver, A. M. *et al.* (1999) IgM<sup>high</sup>CD21<sup>high</sup> lymphocytes enriched in the splenic marginal zone generate effector cells more rapidly than the bulk of follicular B cells. *J. Immunol.* 162(12): 7198–7207.

3. Gommerman, J. L. *et al.* (2000) A role for CD21/CD35 and CD19 in responses to acute septic peritonitis: a potential mechanism for mast cell activation. *J. Immunol.* 165(12): 6915–6921.
4. Roozendaal, R. and Carroll, M. C. (2007) Complement receptors CD21 and CD35 in humoral immunity. *Immunol. Rev.* 219: 157.

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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