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## 1. Description

<b>Components</b>	1 mL monoclonal Anti-IgM antibodies, mouse conjugated to various dyes.	
	FITC	130-095-906
	PE	130-095-908
	APC	130-095-910
	Biotin	130-095-904
<b>Clone</b>	X-54 (isotype: rat Ig2a).	
<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

The Anti-IgM antibody, mouse is suited for the indirect immunofluorescent staining and flow cytometric analysis of cells stained with primary mouse antibodies of IgM isotype. The antibody can further be used for flow cytometric analysis of mouse B cells expressing surface IgM.

### 1.2 Applications

- Identification and enumeration of IgM<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Detection of cells labeled with a primary mouse antibody of IgM isotype.

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-IgM 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.

The antibody is suited for staining of formaldehyd-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) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., Anti-Biotin-PE (# 130-090-756) in combination with Anti-IgM-Biotin.
- (Optional) Primary mouse antibodies of IgM isotype or antibodies for staining of B cells. For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (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).

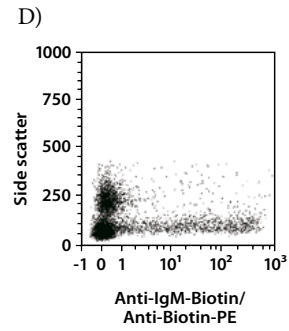
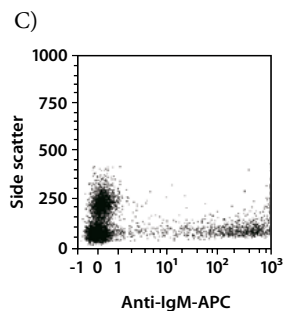
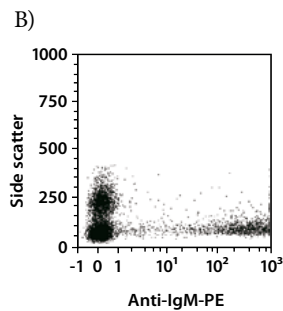
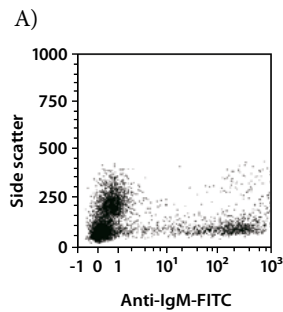
▲ For detection of cells labeled with a primary antibody of mouse IgM isotype, stain cells according to manufacturer's recommendation. After washing proceed with step 2.3.

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 Anti-IgM 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. (Optional) If Anti-IgM-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ 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. Examples of immunofluorescent staining with Anti-IgM antibodies

Human peripheral blood mononuclear cells (PBMCs) were labeled with anti-human CD57 (isotype IgM) and subsequently stained with Anti-IgM antibodies conjugated to FITC (A), PE (B), APC (C), or Biotin (D) and analyzed by flow cytometry using the MACSQuant<sup>®</sup> 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).

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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