

Anti-IgD antibodies human

Anti-IgD-FITC	130-094-552
Anti-IgD-PE	130-094-539
Anti-IgD-APC	130-094-553
Anti-IgD-Biotin	130-094-554
Anti-IgD pure	130-094-555

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 Anti-IgD antibodies

1. Description

Components	1 mL Anti-IgD antibodies, human: monoclonal Anti-IgD antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	IgD26 (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.

1.1 Background information

Anti-IgD antibodies react with the IgD isotype of human immunoglobulins. IgD is a monomeric antibody with two Ig heavy chains of the δ class and two Ig light chains. It is coexpressed with IgM on the surface of mature naive B cells in the periphery. It is also produced in a secreted form that is found in very small amounts in blood serum.

1.2 Applications

- Identification and enumeration of IgD⁺ cells by flow cytometry or fluorescence microscopy.
- Anti-IgD-PE is suited for evaluation of switched memory B cells isolated with the Switched Memory B Cell Isolation Kit, human (# 130-093-617).
- Evaluation of MACS[®] Separations by flow cytometry or fluorescence microscopy. IgD⁺ cells can be isolated by using, for example, the B Cell Isolation Kit II (# 130-091-151) in combination with Anti-IgD-Biotin (# 130-094-554) and Anti-Biotin MicroBeads (# 130-090-485).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-IgD conjugate	FITC	PE	APC	Biotin
Flow cytometry^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11	1:11
- Fixed and permeabilized cells	n. r.	n. r.	n. r.	n. r.

a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.
n. r.: not recommended

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. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-IgD-Biotin.
- (Optional) CD19-FITC (# 130-091-328), CD19-PE (# 130-091-247), or CD19-APC (# 130-091-248). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Mouse IgG1-FITC (# 130-092-213), Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-Biotin (# 130-093-018) for isotype control.
- (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.

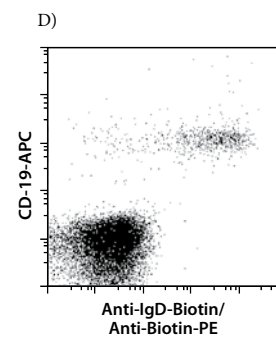
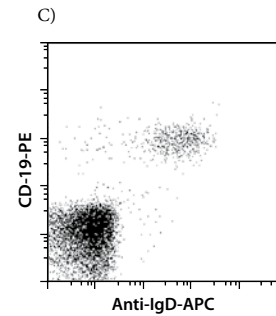
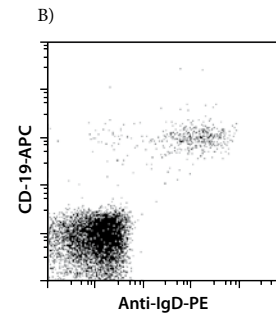
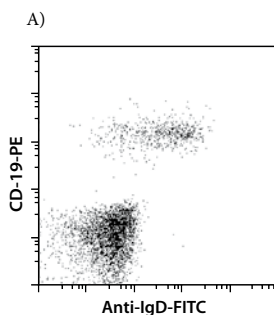
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10^7 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 2×10^7 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 μL of buffer.
4. Add 10 μL of the Anti-IgD antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ\text{C}$).
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
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 Anti-IgD-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), 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-IgD antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgD antibodies conjugated to FITC (A), PE (B), or APC (C), as well as with CD19-PE (# 130-091-247) or CD19-APC (# 130-091-248) and analyzed using the MACSQuant™ Analyzer. Cells labeled with Anti-IgD-Biotin (D) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD19-APC. 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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