



Anti-IgE antibodies human

Anti-IgE-FITC	130-093-065
Anti-IgE-PE	130-093-066
Anti-IgE-APC	130-093-067
Anti-IgE-Biotin	130-093-068
Anti-IgE pure	130-093-127

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-IgE antibodies

1. Description

Components	1 mL Anti-IgE antibodies, human: monoclonal Anti-IgE 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	MB10-5C4 (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-IgE antibodies react with the IgE isotype of human immunoglobulins. IgE is the shortest-lived immunoglobulin with a half-life of two days in serum. Immediate hypersensitivity is triggered by IgE, which binds with high affinity to Fc receptors (FcRs) on mast cells. Antigen-binding to FcR-bound IgE upon re-exposure to specific allergens results in degranulation of mast cells and the release of a variety of mediators like histamine and cytokines. IgE has two main receptors, the high-affinity FcεRI and the low-affinity FcεRII (CD23).

1.2 Applications

- Identification and enumeration of IgE⁺ cells by flow cytometry or fluorescence microscopy.
- Intracellular staining of IgE-producing cells.
- Staining of Fc receptor-bound immunoglobulins.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-IgE conjugate	FITC	PE	APC	Biotin
Flow cytometry^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.

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.
- (Optional) 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-IgE-Biotin.
- (Optional) CD123-PE (# 130-090-899) or CD123-APC (# 130-090-901).
- (Optional) Propidium iodide (PI) 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. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-IgE antibody.

140-093-043-01



5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °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 per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

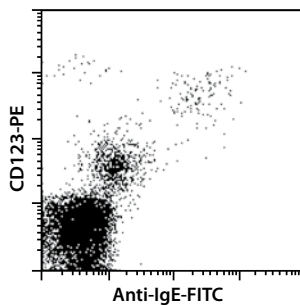
7. (Optional) If Anti-IgE-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.

8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

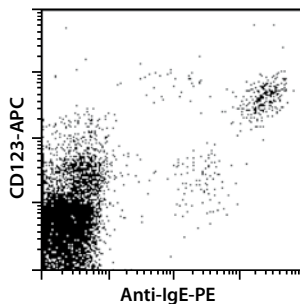
3. Examples of immunofluorescent staining with Anti-IgE antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgE antibodies conjugated to FITC (a), PE (b), or APC (c), as well as with CD123-PE or CD123-APC, and analyzed by flow cytometry. Cells stained with Anti-IgE-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-856) as well as with CD123-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

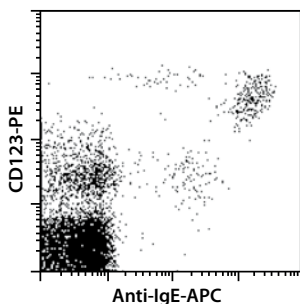
(a) Human PBMCs stained with Anti-IgE-FITC and CD123-PE.



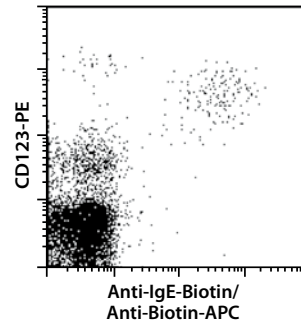
(b) Human PBMCs stained with Anti-IgE-PE and CD123-APC.



(c) Human PBMCs stained with Anti-IgE-APC and CD123-PE.



(d) Human PBMCs stained with Anti-IgE-Biotin, Anti-Biotin-APC, and CD123-PE.



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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

MACS is a registered trademark and autoMACS is a trademark of Miltenyi Biotec GmbH.

© 2008 Miltenyi Biotec GmbH.

140903-04010