

Anti-iNKT antibodies

human

Anti-iNKT-PE	130-094-838
Anti-iNKT-APC	130-094-839
Anti-iNKT-Biotin	130-094-841
Anti-iNKT pure – functional grade	130-094-865

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1. Description

Components	1 mL Anti-iNKT antibodies, human: monoclonal Anti-iNKT antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	6B11 (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. Functional grade antibodies are supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/µg of protein.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

The functional grade product contains no preservative and no stabilizer and is sterile filtered; always handle under aseptic conditions.

1.1 Background information

The Anti-iNKT antibody (clone 6B11) reacts with the T cell receptor (TCR) Va24-Ja18 combined with Vβ11 on human invariant NKT (iNKT) cells.

Natural killer T (NKT) cells represent a distinct lymphocyte population that co-expresses T cell and NK cell surface markers. A subset of human NKT cells, referred to as iNKT cells, expresses an invariant TCR α-chain with certain TCR β-chains (Va24-Ja18 combined with Vβ11). The iNKT cells are implicated in immunoregulatory processes such as tolerance, host defense, and tumor surveillance.^{1,2}

1.2 Applications

- Identification and enumeration of iNKT cells by flow cytometry or fluorescence microscopy.

- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy.
- The Anti-iNKT pure – functional grade antibody is suited, for example, for activation and expansion of iNKT cells.¹

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-iNKT conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11
- Anti-iNKT MicroBead-labeled cells	1:11	1:11	1:11

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.

- Cross-reactivity: The Anti-iNKT antibody is tested to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) 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. 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) Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-iNKT-Biotin.
- (Optional) Anti-iNKT MicroBeads (# 130-094-842) for magnetic labeling.
- (Optional) CD3-PE (# 130-091-374), CD3-APC (# 130-091-373), or CD161-PE (# 130-092-677), and CD45-FITC (# 130-080-202). For more information about fluorochrome-conjugated antibodies see 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 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).

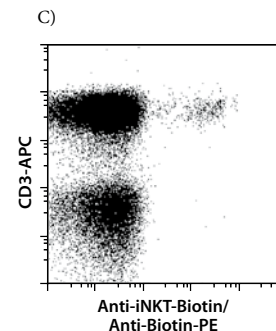
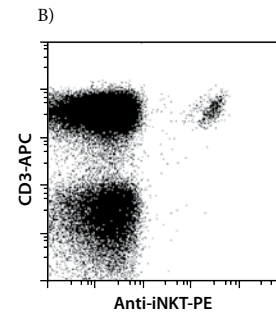
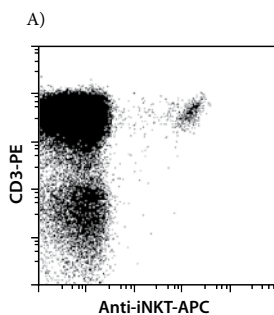
1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the Anti-iNKT antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ \text{C}$).

▲ **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-iNKT-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (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-iNKT antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-iNKT antibodies conjugated to APC (A) or PE (B), as well as with CD3-PE (# 130-091-374) or CD3-APC (# 130-091-373) and CD45-FITC (# 130-080-202), and gated on CD45⁺ leucocytes. Cells labeled with Anti-iNKT-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD3-APC and CD45-FITC. Cells were analyzed 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. Exley, M. *et al.* (2008) Selective activation, expansion, and monitoring of human iNKT cells with a monoclonal antibody specific for the TCR α -chain CDR3 loop. *Eur. J. Immunol.* 38: 1756–1766.
2. Montoya, J.C. *et al.* (2007) Characterization of human invariant natural killer T subsets in health and disease using a novel invariant natural killer T cell-clonotypic monoclonal antibody, 6B11. *Immunology* 122: 1–14.

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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