Anti-iNKT antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
Anti-iNKT-PE	for 30 tests	130-098-128
Anti-iNKT-PE	for 100 tests	130-094-838
Anti-iNKT-APC	for 30 tests	130-098-130
Anti-iNKT-APC	for 100 tests	130-094-839
Anti-iNKT-PE-Vio770	for 30 tests	130-104-148
Anti-iNKT-PE-Vio770	for 100 tests	130-104-110
Anti-iNKT-Biotin	for 30 tests	130-098-131
Anti-iNKT-Biotin	for 100 tests	130-094-841

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.

Technical data and background information

Antigen	iNKT
Clone	6B11
Isotype	mouse IgG1
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	CD22, SIGLEC-2
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*)
Distribution of antigen	NKT cells
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

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 (Vα24-Jα18 combined with Vβ11). The iNKT cells are implicated in immunoregulatory processes such as tolerance, host defense, and tumor surveillance.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

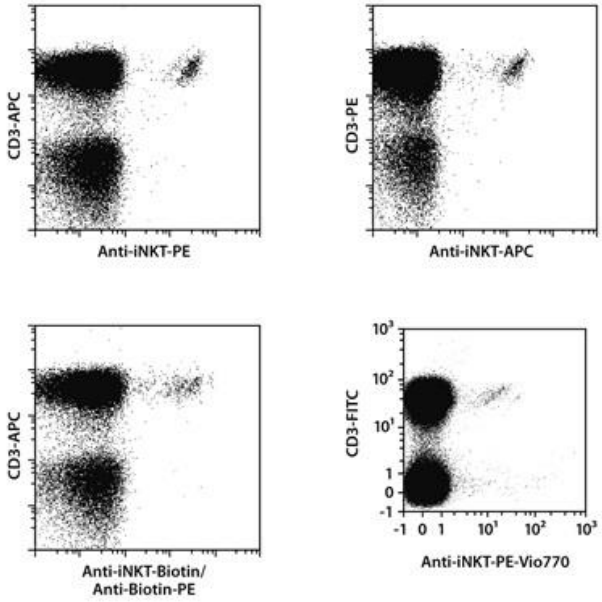
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- 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).

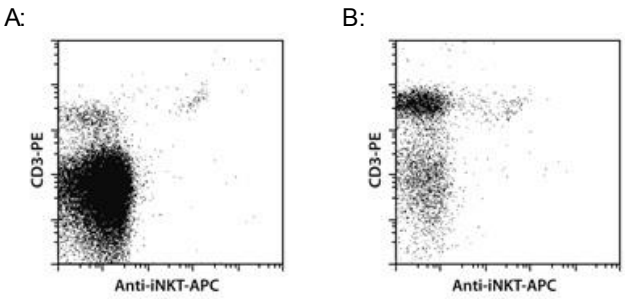
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated 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.

Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-iNKT antibodies as well as with CD3 and CD45 antibodies, and gated on viable $CD45^+$ leucocytes. Cells were 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.



Rhesus monkey PBMCs (A) or cynomolgus monkey PBMCs (B) were stained with Anti-iNKT-APC as well as with CD3-PE and CD45-FITC and analyzed using the MACSQuant® Analyzer. Cells were gated on $CD45^+$ viable leucocytes.



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.

Warranty

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