Anti-IFN-α antibodies
human

Anti-IFN-α-FITC	130-092-600
Anti-IFN-α-PE	130-092-601
Anti-IFN-α-APC	130-092-602
Anti-IFN-α pure	130-092-604

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1. Description

Components	1 mL Anti-IFN-α antibodies, human: monoclonal Anti-IFN-α antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), or allophycocyanin (APC). The unconjugated (pure) antibody is supplied at a concentration of 100 μg/mL.
Clone	LT27:295 (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

Type 1 interferons (IFNs) comprise the gene products of the IFN alpha (IFN-α) and beta (IFN-β) cytokine gene family, of which 12 exist for IFN-α and one for IFN-β. Although first described for its potent anti-viral function, IFN-α also exerts a variety of immunomodulatory effects, including both positive autocrine and paracrine regulation of its own production, the regulation of IL-12 production, the promotion of natural killer (NK) cell–mediated toxicity, and the up-regulation of IFN-γ production in T cells.

IFN-α is predominantly produced by $CD123^+ CD11c^- CD303^+$ plasmacytoid dendritic cells (PDCs).

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High serum levels of IFN-α is a common observation in patients suffering from systemic lupus erythematosus (SLE), having potentially significant effects on the pathogenic perpetuation of the autoimmune nature of the disease.[1]

Clone LT27:295 recognizes the majority of the IFN-α subtypes, but not IFN-α2b.[2]

1.2 Applications

- Identification and enumeration of IFN-α–producing cells, including PDCs.
- Analysis of cytokine production of rare cells by solid-phase staining technology, in combination with magnetic enrichment of cells (see protocol 2.3.2).

1.3 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 Ca^{2+} or Mg^{2+} are not recommended for use.
- Culture medium, e.g., RPMI 1640 containing 10% fetal bovine serum (FBS).
- Reagents for cell culture and stimulation, e.g., IL-3 and CpG ODN2216 for the stimulation of human PBMCs or PDCs.
- Secretion inhibitor, e.g., brefeldin A.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) CD303 (BDCA-2)-FITC (# 130-090-510), CD303 (BDCA-2)-PE (# 130-090-511), or CD303 (BDCA-2)-APC (# 130-090-905). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells.
- (Optional)Plasmacytoid Dendritic Cell Isolation Kit, human (# 130-092-207).
- (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.

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Additional requirements for intracellular cytokine staining in combination with magnetic cell separation (see protocol 2.3.2)

- MACS MicroBeads of choice.
- MS Columns and suitable MACS Separator (MiniMACS™, OctoMACS™, VarioMACS™, or SuperMACS™ Separator).
  ▲ **Note:** Column adapters are required to insert certain columns into VarioMACS or SuperMACS. For details, see MACS Separator data sheets.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocols

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details see the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

2.2 Protocol for *in vitro* stimulation of PBMCs

▲ A **positive control** should be included in the experiment, such as a sample stimulated with CpG ODN2216.

▲ Always include a **negative control** in the experiment. The sample should be treated in exactly the same manner as the stimulated sample but without the addition of stimulants.

1. Wash cells by adding medium, centrifuge at 300×g for 10 minutes.
2. Resuspend cells at a density of 5×10^6 per mL in RPMI 1640 containing 10% FBS, 100 ng/mL IL-3, and 5 µg/mL CpG ODN2216. Plate cells in dishes at a density of 2.5×10^6 cells/cm^2.
   ▲ **Note:** The negative control should be cultivated in culture medium containing FBS and IL-3 only.
3. Incubate cells for 3 hours at 37 °C and 5% CO_2.
4. Add 5 µg/mL brefeldin A and incubate for an additional 3 hours at 37 °C and 5% CO_2.
5. Collect cells carefully by pipetting up and down when working with smaller volumes. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.

2.3 Intracellular immunofluorescent cytokine staining protocols

2.3.1 Intracellular staining of cells in suspension

▲ Volumes for fluorescent labeling 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. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Counterstain cell surface antigens that are sensitive to fixation according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend 10^7 cells in 500 µL of buffer.
4. Add 500 µL of Inside Fix. Mix well and incubate for 20 minutes at room temperature.
5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
   ▲ **Note:** Fixed cells may be stored at 2–8 °C for up to 1 week.
7. (Optional) Counterstain cell surface antigens with antibodies that are sensitive to permeabilization according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
9. Resuspend cells in 90 µL of Inside Perm. Add 10 µL of the Anti-IFN-α antibody.
10. (Optional) Add additional staining antibodies to the solution, e.g. for the staining of cell surface antigens internalized upon cell activation.
11. Mix well and incubate for 10 minutes in the dark at room temperature.
12. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric data acquisition.
    ▲ **Note:** Samples may be stored at 2–8 °C in the dark for up to 24 hours.
    ▲ **Note:** Do not use propidium iodide (PI) or 7-AAD staining.

2.3.2 Intracellular staining in combination with magnetic cell separation (solid phase intracellular staining)

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total 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 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through a 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

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▲ Always wait until the column reservoir is empty before proceeding to the next step.

1. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
3. Add 20 µL of MACS MicroBeads per 10^7 total cells.

   ▲ **Note:** For details on the procedure refer to the respective MACS MicroBead data sheet.
4. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
5. (Optional) Counterstain cell surface antigens with antibodies that are sensitive to fixation according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cells in 500 µL of buffer.
7. Place MS Column in the magnetic field of a suitable MACS Separator.
8. Prepare column by rinsing with 500 µL of buffer.
9. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
10. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 9.

    ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
11. Remove column from the separator and place it on a suitable collection tube.
12. Pipette 500 µL of buffer onto the column. Immediately flush out magnetically labeled cells by firmly pushing the plunger into the column.
13. Add 500 µL of Inside Fix to the eluted cell fraction and incubate for 20 minutes at room temperature.
14. Place a second MS Column in the magnetic field of a suitable MACS Separator and prepare column by rinsing with 500 µL of buffer.
15. Apply the fixed cell suspension onto the column.
16. Wash cells by rinsing the column with 1×500 µL of buffer, followed by 2×500 µL of Inside Perm.
17. Prepare a solution of 10 µL of Anti-IFN-α antibodies and 90 µL of Inside Perm.
18. (Optional) Add additional staining antibodies to the solution, e.g., for the staining of cell surface antigens internalized upon cell activation or antigens which accumulate in the cell.

    ▲ **Note:** Do not exceed the total solution volume of 150 µL.
19. Apply the solution onto the column and incubate for 10 minutes at room temperature.

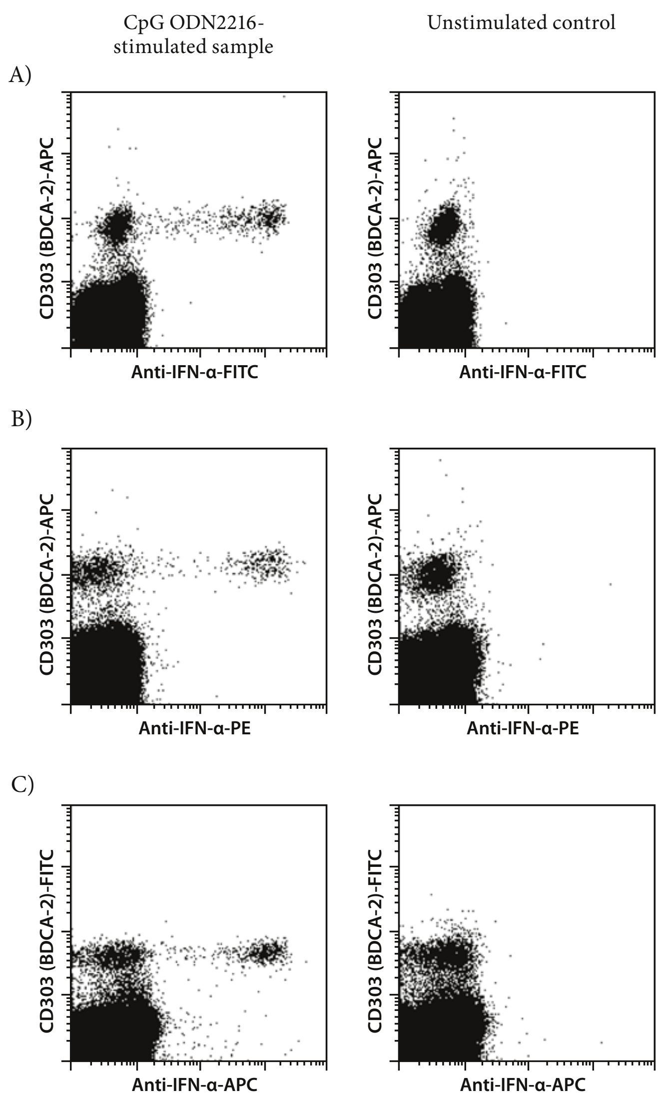
    ▲ **Note:** The MACS Column has a flow-stop mechanism that will retain the solution in the column.
20. Wash cells by rinsing the column with 2×500 µL of Inside Perm followed by 1×500 µL of buffer.
21. Remove column from the separator and place it on a suitable collection tube.
22. Pipette 500 µL of buffer onto the column. Immediately flush out magnetically labeled cells by firmly pushing the plunger into the column.
23. Cells are now ready for analysis. Store cells at 2−8 °C in the dark until analysis. Mix well before flow cytometric data acquisition.

    ▲ **Note:** Samples may be stored at 2−8 °C in the dark for up to 24 hours.

    ▲ **Note:** Do not use propidium iodide (PI) or 7-AAD staining.

3. Examples of immunofluorescent staining with Anti-IFN-α antibodies

Human PBMCs were incubated with or without CpG ODN2216 for 6 hours. After 3 hours, brefeldin A was added. Cells were then fixed, permeabilized, and intracellularly stained with Anti-IFN-α antibodies conjugated to FITC (A), PE (B), or APC (C), as described and analyzed by flow cytometry. Cell surface staining was performed with CD303 (BDCA-2)-APC or CD303 (BDCA-2)-FITC.



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4. References

1. Ronnblom, L. and Alm, G. V. (2001) An etiopathogenic role for the type I IFN system in SLE. Trends Immunol. 22: 427–431.
2. Båve, U. *et al.* (2003) FcγRIIa is expressed on natural IFN-α–producing cells (plasmacytoid dendritic cells) and is required for the IFN-α production induced by apoptotic cells combined with lupus IgG. J. Immunol. 171: 3296–3302.

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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