Anti-IFN-γ antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
Anti-IFN-γ-FITC	9 µg in 300 µL	130-109-768
Anti-IFN-γ-FITC	30 µg in 1 mL	130-109-721
Anti-IFN-γ-PE	9 µg in 300 µL	130-109-769
Anti-IFN-γ-PE	30 µg in 1 mL	130-109-722
Anti-IFN-γ-APC	9 µg in 300 µL	130-109-770
Anti-IFN-γ-APC	30 µg in 1 mL	130-109-723

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	IFN-γ
Clone	REA638
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	Gamma-interferon, IFN-gamma, IFNg
Molecular mass of antigen [kDa]	16
Distribution of antigen	T cells, B cells, NK cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA638 recognizes the mouse interferon-γ (IFN-γ) antigen, a secreted protein which is produced by activated T, B, and NK cells. IFN-γ is a pleiotropic cytokine responsible for macrophage activation and differentiation. It induces transcription of several proinflammatory genes, such as inducible NO synthase, cyclooxygenase-2, and IL-1β, as well as MHC proteins. IFN-γ, in synergy with other cytokines such as TNF-α, inhibits proliferation of normal and transformed cells. Immunomodulatory effects of IFN-γ are exerted on a wide range of cell types expressing the high affinity receptors for IFN-γ. Additional information: Clone REA638 displays negligible binding to Fc receptors.

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.
- Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells containing Inside Fix and Inside Perm.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Protocol for intracellular staining of cells

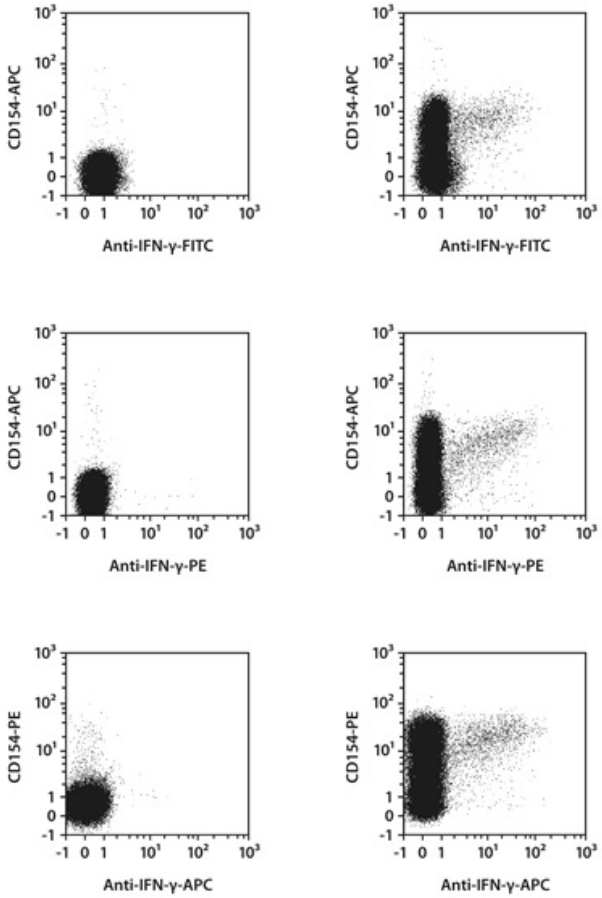
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).
- The special protocol “Intracellular staining in combination with magnetic cell separation” is available for download at www.miltenyibiotec.com/protocols. In-column intracellular staining of cells immobilized on an MS Column is especially advantageous for the analysis of rare cells.

1. Wash up to 10^6 cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens that are sensitive to fixation with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 cells in 250 µL of buffer.
4. Add 250 µL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes in the dark 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 in azide-containing buffer at 2–8 °C for up to 1 week.
7. (Optional) Stain cell surface antigens that are sensitive to permeabilization with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit) and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
9. Resuspend cells in 45 µL of Inside Perm. Add 5 µL of the antibody.
   Note: For staining with several antibodies in this step, reduce the volume of Inside Perm accordingly. For efficient permeabilization, the volume of Inside Perm should be at least 30% of the overall staining volume.
10. Mix well and incubate for 10 minutes in the dark at room temperature.
11. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
12. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of Inside Perm, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 10 and 11.
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 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.

Examples of immunofluorescent staining

Splenocytes from BALB/c mice, either left unstimulated (left images) or stimulated with CD3/CD28 antibodies and1 µg/mL brefeldin A, were fixed and permeabilized. Cells were then stained with Anti-IFN-γ antibodies as well as with CD154 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. $CD4^+$ cells were pre-gated for the analysis. Cell debris were excluded from the analysis based on scatter signals.



References

1. **Gray, P. W. *et al.*** (1983) Cloning and expression of murine immune interferon cDNA. Proc. Natl. Acad. Sci. U.S.A. 80(19): 5842–5846.
2. **Chan, E. D. *et al.*** (2001) IFN-gamma + LPS induction of iNOS is modulated by ERK, JNK/SAPK, and p38(mapk) in a mouse macrophage cell line. Am. J. Physiol., Cell Physiol. 280(3): 441–450.
3. **Vila-del Sol, V. *et al.*** (2008) IFN-gamma-induced TNF-alpha expression is regulated by interferon regulatory factors 1 and 8 in mouse macrophages. J. Immunol. 181(7): 4461–4470.

Warranty

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