Product Information

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Monoclonal Anti-Interferon-γ–Carboxyfluorescein
Clone 25723.11
produced in mouse, purified immunoglobulin

Catalog Number **F8648**

Product Description

Monoclonal Anti-Interferon-γ–Carboxyfluorescein (mouse IgG2b isotype) is derived from the hybridoma produced by the fusion of a mouse myeloma cell line and splenocytes from a mouse immunized with human recombinant IFN-γ. The product is prepared by conjugation of carboxyfluorescein to Protein G purified IFN-γ monoclonal antibody.

Human Interferon-γ (IFN-γ) is a potent modulator of immune response and cellular processes. IFN-γ exerts a wide variety of biological effects including anti-viral activity, inhibition of cell or tumor growth, and promotion of B cell differentiation into plasma cells. IFN-γ acts as a signal for major histocompatibility antigen expression, and is classified as an immune interferon. IFN-γ, together with IL-2, IL-12 and TNF-β, are known as Type I cytokines and promote cell-mediated immunity involving macrophages, monocytes and cytotoxic T cells. This is in contrast to the Type II cytokines (IL-4, IL-5, IL-6, IL-10, IL-13), which accompany humoral or antibody-mediated immunity. IFN-γ functions as an activating factor to primed macrophages (MAF) for non-specific tumoricidal activity and activates monocytes for enhanced cytotoxicity against tumor cells. IFN-γ also boosts the cytotoxicity of natural killer cells and stimulates T cell cytotoxicity. The species specificity of IFN-γ resides in the interaction of IFN-γ with its receptor. Human IFN-γ does not bind specifically to mouse, hamster or bovine cells.

This product is designed to detect intracytoplasmic cytokines and enumerate cytokine-producing cells in a mixed population using flow cytometric assays. The cytokine profile of activated cells has profound implications on the immune status of the host. T helper cells are generally considered as the cellular sources of cytokines. In the original differentiation model for CD4+ T helper cells, precursors were referred to as Th_0 which differentiate into functional subsets designated as Th_1 and Th_2. Th_0 cells secrete a combination of both type I and type II cytokines, whereas Th_1 were thought to secrete only type I (IL-2, IL-12, TNF-β and IFN-γ) cytokines and Th_2 only type II (Il-4, IL-5, IL-6, IL-10, IL-13) cytokines. In recent years additional experimental data have lead to the revision of this model. The cytokine profiles of CD4+ T helper cells are not mutually exclusive as originally envisioned. In addition to CD4+ cells, CD8+ cells are also active in both type I and type II cytokine production. Clearly, the cytokine profile of specific functional cell subsets during immune activation influences the outcome of the response. The ability to manipulate the cytokine production profile holds promise for modulating the balance between Th_1 and Th_2, or type I and type II, cytokine effects towards achieving protective immunity. Such therapeutic potential, as well as the insight into underlying immune mechanisms, has stimulated much research into this area. Intracellular cytokine staining reagents can be used in flow cytometry to correlate cytokine production profiles at the single cell level with cell surface phenotype, e.g., CD4, CD8, activation antigen expression. This approach is much more informative than the traditional methods of quantitation of secreted cytokine accumulated in culture supernatant of functionally heterogeneous cell populations. This method is rapid and avoids imprecise and tedious physical cell enrichment methods.

Reagent

Supplied as a solution in phosphate buffered saline containing 0.1% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C. Protect from prolonged exposure to light. Do Not Freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Paraformaldehyde Fixative:
4.0 grams paraformaldehyde, Catalog Number P6148, in 100 mL sterile PBS, pH 7.4. Dissolve by heating to 56 °C for about 1 hour. Store at 2-8 °C for up to 2 weeks. Protect from light.

Saponin Buffer:
0.1 grams saponin, Catalog Number S4521, dissolved in 100 mL of 1× Hanks Balanced Salt Solution (use 10× HBSS, Catalog Number H4641) with 0.05% sodium azide. Store at room temperature for no longer than one month.

Conditions for Intracellular Cytokine Staining[1-5]

1. Cell preparation
   Enrich peripheral blood for lymphocytes using ficoll. Stimulate lymphocytes *in vitro* with 10 ng/ml PMA (phorbol myristate acetate) and 1 μM calcium ionomycin. To inhibit protein secretion, add 2 μM monensin and culture for 18 hours. Harvest cells and wash in PBS.
2. Cell surface staining
   If cells are to be stained with another monoclonal antibody conjugate, follow the manufacturer's staining procedure.
3. Fixation and permeabilization.
   Fix with cold 4% paraformaldehyde for 10 minutes at room temperature with intermittent vortexing to maintain a single cell suspension. Wash again and permeabilize with 0.1% saponin in buffer.
4. Antibody staining
   To 1-5 x 10^5 cells suspended in ~200 μL saponin buffer, add 10 μL Anti-IFN-γ-Carboxyfluorescein. Protect from light and incubate at 22 °C for 30-45 minutes. Wash cells 2x with saponin buffer. Finally, resuspend cells in PBS containing 1% bovine serum albumin.
5. Detection
   Analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding. The ideal negative control is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

Results

When assayed by flow cytometric analysis, using 10 μL of the antibody conjugate to stain 1-5 x 10^5 cells, the antibody conjugate detects intracellular levels of cytokine in secreting cells.

Note: In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

Specificity

The product is determined to be specific by a group of assays that include:

a. Inhibition of staining in the presence of excess exogenously added IFN-γ.
b. Inhibition of staining in the presence of unconjugated anti-IFN-γ and/or
c. Lack of staining when cells are fixed but not permeabilized

References

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3. Prussin, C., *J. Clin. Immunol*., 17, 195 (1997).
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