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Product Information

Monoclonal Anti-Tumor Necrosis Factor-α-Carboxyfluorescein, Clone 6402.31
antibody produced in mouse, purified immunoglobulin

Catalog Number **F6651**

Product Description

Monoclonal Anti-Tumor Necrosis Factor-α (mouse IgG1 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 TNF-α. The conjugate is prepared by conjugation of carboxyfluorescein to Protein G purified TNF-α monoclonal antibody.

Tumor necrosis factor-α (TNF-α) also known as cachectin, is an inflammatory cytokine, which exists mainly as a 51 kDa complex of three identical, noncovalently linked polypeptide subunits.[1,2] The human subunit contains 157 amino acids with a molecular weight of ~17 kDa. TNF-α occurs as a secreted, soluble form and as a membrane-anchored form, both of which are biologically active. Membrane-bound TNF-α from activated human monocytes appears as a 26 kDa polypeptide translation product and includes an uncleaved 76-residue signal sequence. Although this leader peptide is removed co-translationally to yield the 17 kDa secreted form, it apparently persists in membrane TNF. The naturally occurring form of TNF-α is glycosylated, but non-glycosylated recombinant TNF-α has comparable biological activity. Human and murine TNF-α show ~79% homology at the amino acid level between the two species. Murine cytotoxic T lymphocytes (CTL) appear to express a membrane protein of 50-60 kDa, which is immunologically related to TNF-α.

Two types of receptors for TNF-α have been described and virtually all cell types studied show the presence of one or both of these receptor types. TNF-α shares 30% homology with TNF-β at the DNA level, both bind to the same receptor and have similar biological effects. Many substances can induce the production of TNF, but bacterial cell wall products such as lipopolysaccharides are among the most potent inducers. TNF-β is produced by neutrophils, activated lymphocytes, macrophages, NK cells, LAK cells, astrocytes, endothelial cells, smooth muscle cells, and some transformed cells. TNF-β appears to be a major mediator of inflammatory responses and over production results in the extensive tissue damage associated with endotoxemia and cachexia. High serum levels of TNF are associated with septic shock, rejection of renal transplants, parasitic infections, and various neoplastic diseases. TNF-α influences the growth and function of both normal and neoplastic cells. Using normal cells, TNF-α exhibits growth enhancing activities for fibroblasts, T and B cells, functional activation of endothelial cells and neutrophils, and alterations of the growth and differentiation of myeloid cells.[2] On neoplastic cells, TNF-α exerts growth inhibitory activities that can be either cytostatic or cytotoxic. Cytostasis can reflect the ability of TNF-α to induce cellular differentiation. Because these molecules play major roles in biological responses and can contribute to pathological states, an *in vitro* assay for their detection and quantitation is desirable.[3]

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, and IL-13) cytokines. In recent years, additional experimental data has 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 (CD4, CD8, activation antigen expression etc.). 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. Intracellular cytokine staining can be coupled with the CELL CENSUS PLUS™ System, Catalog Number CCPS1, to correlate the proliferation activity of immune cell subsets with particular cytokine-producing-profile(s) in response to different cell activation stimuli.

Reagent
The conjugate is provided as a solution in saline containing up to 0.5% bovine serum albumin and up to 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 of 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 gram of saponin, Catalog Number S4521, dissolved in 100 ml of 1x Hank's Balanced Salt Solution (10x HBSS, Catalog Number H4641) with 0.05% sodium azide. May be stored at 22 °C for up to one month.

Conditions for Intracellular Cytokine Staining[4-8]
1. Cell preparation
   Enrich peripheral blood for lymphocytes using Ficoll. Stimulate lymphocytes *in vitro* with 10 ng/ml of 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 solution at 22 °C for 10 minutes, with intermittent vortexing to maintain a 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 of saponin buffer, add 10 μL of Anti-TNF-α-Carboxyfluorescein. Protect from light and incubate at 22 °C for 30-45 minutes. Wash cells twice 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.

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding. The ideal negative control reagent 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.

Product Profile
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 TNF-α.
b. Inhibition of staining in the presence of unconjugated anti-TNF-α and/or
c. Lack of staining when cells are fixed but not permeabilized

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.

References

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