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ProductInformation

MONOCLONAL ANTI-HUMAN INTERLEUKIN-6 (IL-6), CLONE 1936.141, FITC CONJUGATE
Purified Mouse Immunoglobulin

Product No. **F 6276**

Product Description

Interleukin-6 is a multifunctional protein originally discovered in the media of cells stimulated with double stranded RNA.[1] IL-6 appears to be directly involved in the responses that occur after infection and injury and may prove to be as important as IL-1 and TNF-α in regulating the acute phase response.[2,3] IL-6 is reported to be produced by fibroblasts, activated T cells , activated monocytes or macrophages, and endothelial cells. It acts upon a variety of cells, including fibroblasts, myeloid progenitor cells, T cells, B cells, and hepatocytes. IL-6 induces multiple effects, as indicated by its numerous synonyms: plasmacytoma growth factor (PCT-GF), interferon-β_2, monocyte derived human B cell growth factor, B cell stimulating factor (BSF-2), hepatocyte stimulating factor (HSF), interleukin hybridoma/plasmacytoma-1 (IL-HP1). IL-6 also interacts with IL-2 in the proliferation of T lymphocytes.[5] IL-6 also potentiates the proliferative effect of IL-3 on multipotential hematopoietic progenitors.[4]

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 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 (Product Code CCPS-1) to correlate the proliferation activity of immune cell subsets with particular cytokine-producing-profile(s) in response to different cell activation stimuli.

Reagents

Monoclonal Anti-Human IL-6 (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 IL-6. The product is prepared by conjugation of carboxyfluor- escein (FITC) to Protein A purified IL-6 monoclonal antibody. The conjugate is provided as a 0.2 µm filtered solution in 0.01 M HEPES buffered saline, pH 7.2, containing 0.5% bovine serum albumin and 0.1% sodium azide as preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous 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 (Product No. P6148) in 100 ml sterile PBS, pH 7.4. Dissolve by heating to 56 °C for about 1 hour. Store 2-8 °C for up to 2 weeks. Protect from light.
Saponin Buffer:
0.1 grams saponin (Product No. S4521) dissolved in 100 ml of 1X Hank's Balanced Salt Solution (10X HBSS, Product No. H4641) with 0.05% sodium azide. Store at 22 °C for up to one month.

Conditions for Intracellular Cytokine Staining[5-9]
1. Cell preparation
Enrich peripheral blood for mononuclear cells using ficoll. Stimulate lymphocytes *in vitro* with 100 ng/ml LPS (lipopolysaccharide). To inhibit protein secretion, add 2 µM monensin and culture for 6 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 at 22 °C for 10 minutes, 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 approximately 200 µl saponin buffer, add 10 µl Anti-IL-6 -FITC conjugate. Protect from light and incubate at 22 °C for 30-45 minutes. Wash cells 2 x 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 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.

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 IL-6.
b. Inhibition of staining in the presence of unconjugated anti-IL-6 and/or
c. Lack of staining when cells are fixed but not permeabilized

References

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