

## Product Information

### Monoclonal Anti-Interleukin-4–R-Phycoerythrin Clone 3007.11

produced in mouse, purified immunoglobulin

Catalog Number **P7097**

Synonym: Monoclonal Anti-IL-4

#### Product Description

Interleukin-4 (IL-4) is a lymphokine that has profound effects on the growth and differentiation of immunologically competent cells.<sup>1</sup> Synonyms for IL-4 include: B cell stimulatory factor-1 (BSF-1), T cell growth factor-2 (TCGF-2), and mast cell growth factor-2 (MCGF-2).<sup>2-4</sup> Interleukin-4 is a complex glycoprotein released by a subset of activated T cells. The molecular mass of IL-4 occurring naturally is 12–20 kDa.

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<sub>0</sub>, which differentiate into functional subsets designated as Th<sub>1</sub> and Th<sub>2</sub>. Th<sub>0</sub> cells secrete a combination of both type I and type II cytokines; whereas, Th<sub>1</sub> were thought to secrete only type I (IL-2, IL-12, TNF-β, and IFN-γ) cytokines and Th<sub>2</sub> only type II (IL-4, IL-5, IL-6, IL-10, IL-13) cytokines. In recent years additional experimental data have led 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<sub>1</sub> and Th<sub>2</sub> 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.

#### Reagent

Monoclonal Anti-Human IL-4 (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 IL-4. The product is prepared by conjugation of R-phycoerythrin (PE) to Protein G purified IL-4 monoclonal antibody.

The conjugate is provided as 25 µg of antibody in 1.0 mL of saline containing up to 0.5% BSA. Contains 0.1% sodium azide as a preservative.

#### 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

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.

Paraformaldehyde Fixative - 4.0 g of paraformaldehyde (Catalog Number P6148) in 100 ml sterile PBS, pH 7.4. Dissolve by heating to 56 °C for ~1 hour. Store 2–8 °C for up to 2 weeks. Protect from light.

Saponin Buffer - 0.1 grams of saponin (Catalog Number S4521) dissolved in 100 ml of 1× Hanks' Balanced Salt Solution (10× HBSS, Catalog Number H4641) with 0.05% sodium azide. Store at 22 °C for up to one month.

### Conditions for Intracellular Cytokine Staining<sup>5-9</sup>

1. Cell preparation - Enrich peripheral blood for lymphocytes using CD4+ cell selection columns. Stimulate *in vitro* with 10 ng/ml PMA (phorbol myristate acetate), 1 μM calcium ionomycin and 5 μg/ml PHA. To inhibit protein secretion, add 2 μM monensin and culture for 5 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 × 10<sup>5</sup> cells suspended in ~200 μl saponin buffer, add 10 μl of Anti-IL-4 -PE conjugate. 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.

## Results

When assayed by flow cytometric analysis, using 10 μl of the antibody conjugate to stain 1–5 × 10<sup>5</sup> 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:

1. Inhibition of staining in the presence of excess exogenously added IL-4.
2. Inhibition of staining in the presence of unconjugated anti-IL-4 and/or
3. Lack of staining when cells are fixed but not permeabilized.

## References

1. Howard, M., et al., *J. Exp. Med.*, **155**, 914 (1982).
2. Mosmann, T., et al., *Proc. Nat. Acad. Sci. USA*, **83**, 5654 (1986).
3. Paul, W., and Ohara, J., *Ann. Rev. Immunol.*, **5**, 429 (1987).
4. Park, L., et al., *J. Exp. Med.*, **166**, 476 (1987).
5. Jung, T., et al., *J. Immunol. Methods*, **159**, 197 (1993).
6. Prussin, C., and Metcalfe, D.D., *J. Immunol. Methods*, **188**, 117 (1995).
7. Prussin, C., *J. Clin. Immunol.*, **17**, 195 (1997).
8. Estcourt, C., *Clin. Immunol. Immunopathol.*, **83**, 60 (1997).
9. Ito, M., et al., *Clin. Immunol. Immunopathol.*, **83**, 281 (1997).

ANK,PHC 01/14-1