

**PN IM2719** IL-4 - PE

100 tests  
20 µL/test

(4D9)



**IOTest®**  
Conjugated Antibodies

For Research Use Only. Not for use in diagnostic procedures.

#### SPECIFICITY

Interleukine 4 (IL-4), is a monomeric protein with two potential N-linked glycosylation sites. The level of glycosylation is responsive for molecular weight (Mr) heterogeneity of the molecule ranging from 15 to 19 kDa (1).

The biological effects of IL-4 are mediated by the binding to its specific receptors (IL-4R). The IL-4R is composed of at least two chains, the  $\alpha$  chain (CD124) and the  $\gamma$  chain also found in other cytokines receptors (i. e. the IL-2R, IL-7R, IL-9R, IL-13R and IL-15R) (1).

IL-4 is produced by a subset of activated hematopoietic cells, including T lymphocytes, mast cells and basophils (2,3). IL-4 was initially named BSF-1 (B Cell Stimulating Factor -1), BCGF (B Cell Growth Factor) and BCDF (B Cell Differentiation Factor): In fact, IL-4 participates in the activation, proliferation and differentiation of B lymphocytes. Together with IL-13, IL-4 is an inducer of B-lymphocytes switching IgE production and probably particular (non-complement-fixing) IgG isotypes (4,5). IL-4 can affect a variety of target cells such as T lymphocytes, monocytes, endothelial cells and fibroblasts (1,3).

IL-4 is also involved in Th1 - Th2 (T helper 1 - T helper 2) cytokine pathways regulating Th2 cells as an autocrine growth factor (3,2,4, 6,7,8). The cytokines produced by Th1 and Th2 lymphocyte subsets (CD4+ lymphocytes) determine a symmetrical pathway of the immune response. Activated CD4+ lymphocytes of the Th1 profile secrete IL-2, IFN $\gamma$  (Interferon  $\gamma$ ) and TNF $\beta$  (Tumor Necrosis Factor  $\beta$ ): they are reported to be involved in cellular immunity and delayed type hypersensitivity reactions (DHT). Activated CD4+ lymphocytes of the Th2 profile produce IL-4, IL-5, IL-6 and IL-10. Th2 profile of cytokines secretion is known to be responsive for humoral immune responses, allergy, enhancement of antibody production (particularly IgE response), and promotion of eosinophils proliferation and function (6). Th1 and Th2 pathways each enhances the development of cells pertaining to the same subset while suppress the expansion and / or effector functions of the other subset (9,5,10).

Th1- or Th2- cytokines profile is not specifically produced by Th lymphocytes, but also by Tc lymphocytes allowing to generalize the nomenclature (7, 11): Th1- or Th2-like cytokines profile may be termed Type 1 or Type 2 response (6,7,9,5).

#### REAGENT

**Clone** 4D9  
**Isotype** IgG1 mouse  
**Source** Serum-free culture supernatant  
**Purification** Affinity chromatography  
**Conjugation** PE: R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of IgG.  
**Excitation wavelength:** 488 nm  
**Maximum emission wavelength:** 575 nm  
**Main emission color:** Orange-red  
**Buffer** 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

#### APPLICATION

Flow cytometric analysis of intracellular cytokines.  
Research studies of intracellular cytokines production from lymphocyte subsets.  
Research studies of Type1 / Type 2 lymphocyte subsets during the immune response.  
Research studies of lymphocyte subset activation and / or function.

**NOTE:** Analysis may require the in vitro stimulation of the cells of interest in the presence of brefeldin A (BFA) and / or monensin blocking the cytokine secretion in the Golgi complex (4,9,12,13).

The activated cells are then permeabilized in order to detect intracellular accumulation of IL-4 using anti-IL-4 conjugated monoclonal antibody as probe (14,8,15). The PE-conjugated form of the 4D9 clone is optimized to detect intracellular IL-4 after formaldehyde / saponin-based permeabilizing procedures on whole blood and peripheral mononuclear cells (PBMC) (7, 8). IntraPrep™ Permeabilization Reagent is recommended (PN IM2388, PN IM2389).

Conjugated isotypic control, or use of excess of unconjugated anti-IL-4 as blocking antibody, or pre-incubation of molar excess of recombinant human IL-4 prior to staining, or unstimulated cells may be used as a control test of the intracellular cytokines staining.

#### STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Lithium / Heparin is required as anticoagulant to collect samples for intracytoplasmic detection of cytokines in "in vitro" stimulation studies.

#### STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8° C. Do not freeze. Minimize exposure to light.

#### REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20 - 25° C prior to use.

#### PROCEDURE

This reagent is designed for Flow Cytometry. Permeabilization procedure using IntraPrep™ Permeabilization Reagent (PN IM2388, PN IM2389) Combined membrane and intracytoplasmic staining procedure using conjugated antibodies:

1. Dispense 50 µL of whole blood (or  $5 \times 10^5$  WBC) into two tubes for each sample:
  - 1 membrane and cytoplasmic staining test tube = assay tube
  - 1 membrane and cytoplasmic control tube (isotypic control or specific antibody used for the control tube) = control tube
2. Add
  - 20 µL (or 10 µL, see IOtest labeling procedure) of membrane-specific conjugated monoclonal antibody to each assay tube and
  - 20 µL (or 10 µL, see IOtest labeling procedure) of appropriate membrane isotypic control (or conjugated specific antibody used for the control tube) to each control tube.

1/3



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- Vigorously vortex tube by tube.
  - Incubate for 15 minutes at room temperature (18 - 25° C) in the dark.
  - Add 100 µL of IntraPrep Reagent 1 to each tube.
  - Vigorously vortex tube by tube.
  - Incubate for 15 minutes at room temperature (18 - 25° C) in the dark.
  - Add 4 mL of PBS.
  - Centrifuge for 5 minutes at 300 x g at room temperature (18 - 25° C).
  - Discard supernatant (by aspiration).
  - Add 100 µL of IntraPrep Reagent 2 to each tube. Let mixing occurs WITHOUT VORTEXING.
  - Incubate for 5 minutes at room temperature (18 - 25° C) WITHOUT VORTEXING.
  - Gently agitate (manually), for 1 to 2 seconds.
  - Add 20 µL (or 10 µL, see IOTest labeling procedure) of intracellular conjugated specific antibody to each assay tube and 20 µL (or 10 µL, see IOTest labeling procedure) of the appropriate intracellular control reagent (e. g. conjugated isotopic control or conjugated specific antibody used for the control tube) to each control tube.
  - Gently vortex tube by tube.
  - Incubate for 15 minutes at room temperature (18 - 25° C) in the dark.
  - Add 4 mL of PBS.
  - Centrifuge for 5 minutes at 300 x g at room temperature (18 - 25° C).
  - Discard supernatant (by aspiration).
  - Resuspend cells in 500 µL of PBS, containing 0.5% formaldehyde and proceed to flow cytometry analysis.
- The specimen should be analyzed within two hours of IntraPrep treatment when stored at 18-25° C. Otherwise, fixed preparations should be stored at 2 - 8° C in the dark and analyzed within 24 hours.

If the membrane staining is not required, start the permeabilization procedure at the step 5 by adding 100 µL of IntraPrep Reagent 1 to 50 µL of whole blood (or 5 x 10<sup>5</sup> cells/test) and continue as described.

### EXAMPLE DATA

The graphs below are biparametric representations (Fluorescence Intensity versus Fluorescence Intensity) of activated (graphs 1 - 2) and non-activated (graphs 3 - 4) normal human whole blood sample. "in vitro" activation is with PMA and Ionomycin in the presence of Brefeldin A and Monensin. Heparinized whole blood sample (e. g. 50 µL) is activated (or non-activated) during 4 hours with an equivalent (e. g. 50 µL) volume of a 2X "activation-solution" \* (or "non-activation solution" \*\*)

Membrane staining is with IOTest CD4-FITC and CD3-PC5 (respectively PN IM0448 and PN IM2635). Intracellular staining is with IL-4-PE (PN IM2719) gated on CD3+ lymphocytes. Permeabilization is with IntraPrep Permeabilization Reagent (PN IM2388).

\* Composition of the 2X "activation solution": RPMI 1640 Medium without L-Glutamine: Boehringer Ingelheim Bioproducts Ref. 12-167 F, FCS: 10% HyClone Ref. SH30080, L-Glutamine: 2mM Sigma Ref. G7513, Non-Essential Amino Acid Solution: 0.1 mM Sigma-Aldrich Ref. M7145, Sodium Pyruvate Solution: 1 mM Sigma-Aldrich Ref. S8636, PMA: 40 ng/mL Calbiochem Novabiochem Ref. 524400, Ionomycin: 4 µg/mL Sigma Ref. I-0634, Brefeldin A:

40 µg/mL Sigma Ref. B-7651 and Monensin: 2µM Calbiochem Novabiochem Ref. 475896.

\* Composition of the 2X "Non-activation solution": RPMI 1640 Medium without L-Glutamine: Boehringer Ingelheim Bioproducts Ref. 12-167 F, FCS: 10% HyClone Ref. SH30080, L-Glutamine: 2mM Sigma Ref. G7513, Non-Essential Amino Acid Solution: 0.1 mM Sigma-Aldrich Ref. M7145, Sodium Pyruvate Solution: 1 mM Sigma-Aldrich Ref. S8636, Brefeldin A: 40 µg/mL Sigma Ref. B-7651 and Monensin: 2µM Calbiochem Novabiochem Ref. 475896

### Activated blood sample

-Upper left quadrant (1) contains CD4 negative / IL-4 positive events representing CD4 negative lymphocytes which may produce IL-4 under appropriate "in vitro" activation.

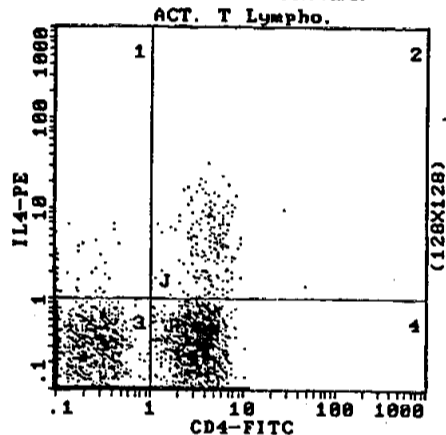
-Upper right quadrant (2) defines double positive CD4+ / IL-4+ T lymphocytes representing T "helper" lymphocytes producing IL-4 under appropriate "in vitro" activation.

-Lower left quadrant (3) contains double negative CD4- / IL-4- events among CD3 positive lymphocytes.

-Lower right quadrant (4) contains CD4 positive / IL-4 negative T lymphocytes and represents T "helper" lymphocytes which do not produce IL-4 after "in vitro" activation.

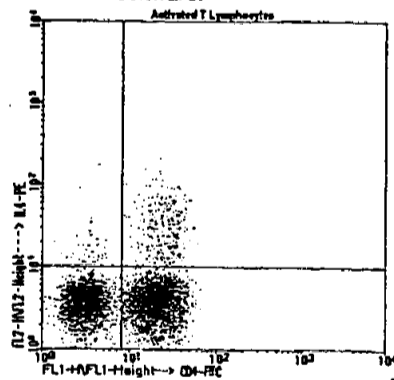
### Graph1

Acquisition is with a COULTER R EPICS R XL flow cytometer. Analysis is with the XL SYSTEM II TM software.



### Graph2

Acquisition is with a Becton Dickinson TM flow cytometer. Analysis is with the LYSYS II TM software.



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2/3

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Manufactured by



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130, avenue de Lattre de Tassigny B.P. 177 13276 MARSEILLE Cedex 9 (FRANCE)  
Tel : (33) 4 91 17 27 00 - Fax : (33) 4 91 41 43 58 - e-mail : abmarket@immunotech.fr

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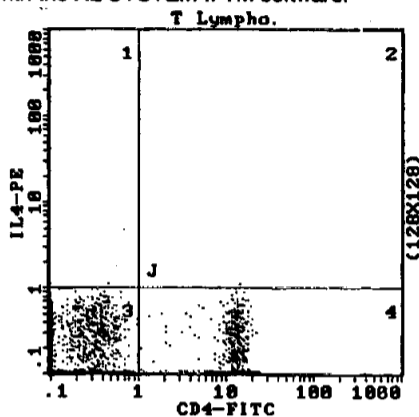
Non-activated whole blood sample:

-Lower left quadrant (3) contains double negative CD4- / IL-4- events among CD3 positive lymphocytes.

-Lower right quadrant (4) contains CD4 positive / IL-4 negative T lymphocytes and represents T "helper" lymphocytes which do not produce IL-4.

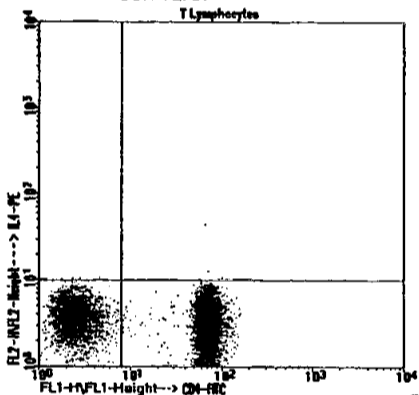
Graph3

Acquisition is with a COULTER R EPICS R XL flow cytometer. Analysis is with the XL SYSTEM II TM software.



Graph4

Acquisition is with a Becton Dickinson TM flow cytometer. Analysis is with the LYSYS II TM software.



138-146.

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3/3



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