



**CD4-APC**  
Ref: CYT-4AP3

*For Research Use Only*

#### **INTENDED USE**

CD4-APC is a monoclonal antibody (MAb) labelled with the Allophycocyanine (APC) designed for use as a direct immunofluorescence reagent in the identification and enumeration of cells which express the CD4 antigen by flow cytometry.

#### **SUMMARY AND EXPLANATION**

Flow Cytometry is a powerful tool for the analytical and quantitative characterization of cells which provides rapid, quantitative and multiparametric analysis of heterogeneous cell populations on a cell-by-cell basis. Flow cytometry is performed on cells in liquid suspension that have been incubated with fluorescently-labeled antibodies directed against specific cellular proteins. The relative fluorescence intensity of the positive cells indicates the amount of antibody bound to specific binding sites on the cells, and therefore provides a relative measure of antigen expression.

Human lymphocytes may be classified in three main populations according to their biological function and their cell surface antigen expression: T lymphocytes, B lymphocytes and natural killer cells (NK). T lymphocytes (CD3+), the precursors of which originate in the bone marrow and then migrate and mature in the thymus, can be subdivided as well in functionally different populations. The most clearly defined of these are helper/inducer T cells (CD3+CD4+) and suppressor/cytotoxic T cells (CD3+CD8+). T cells produce no antibodies and are the mediators of cell immunity.

The CYT-4AP3 MAb recognizes the CD4 antigen present in helper/inducer T cells (CD3+CD4+), and can therefore be used in the characterization studies for immunophenotyping of lymphocytes. These studies are widely applied for monitoring of the immunologic status of post-transplant patients and in the characterization and follow-up of immunodeficiencies, autoimmune diseases, leukemia etc<sup>(1, 2)</sup>. Individuals with HIV typically exhibit a steady decrease of helper/inducer T lymphocyte counts as the infection progresses. The CD4 antigen is also detected on some monocytes.

#### **PRINCIPLES OF THE PROCEDURE**

Flow cytometry (FC) is an innovative technology by means of which different cell characteristics are simultaneously analyzed on a single cell basis. This is achieved by means of hydrodynamic focusing of cells that pass aligned one by one in front of a set of light detectors; at the same time they are illuminated by a laser beam. The interaction of the cells with the laser beam generates signals of two different kinds: those generated by dispersed light (FSC/SSC), which mainly reflects the size of the cell and its internal complexity, and those related to the emission of light by the fluorochromes present in the cell. These signals become electric impulses which are amplified and registered as digital signals to be processed by a computer.

When the reagent is added to the sample, the fluorochrome-labelled MAb presents in the reagent bind specifically to the antigens they are directed against, allowing the detection by FC of the cell populations carried by the antigen.

The erythrocyte population, which could hinder the detection of the target population, is eliminated by the use of a red blood cell lysing solution previous to acquire the sample on the cytometer. The use of Quicklysis™ (CYT-QL-1) erythrocyte lysing solution is recommended, since it requires no further washing step and contains no fixative, therefore minimizing the handling of the sample and avoiding the cell loss associated to the centrifuge process<sup>(3, 4)</sup>.

#### **REAGENT COMPOSITION**

Purified monoclonal CD4 Antibody Allophycocyanine (APC) conjugated, supplied in phosphate buffered saline with 0,1% sodium azide.

Clone: HP2/6

Isotype: IgG2a

Purification: Affinity chromatography

Amount per 1 ml vial: 200 tests (5 µl MAb to 10<sup>6</sup> cells)

Reagents are not considered sterile.

#### **STORAGE CONDITIONS**

The reagent is stable until the expiration date shown on the label, when stored at 2-8°C. The reagent should not be frozen or exposed to direct light during storage or during incubation with cells. Keep the reagent vial dry. Once opened, the vial must be stored in a vertical position to avoid any possible spillage.

#### **WARNINGS AND RECOMMENDATIONS**

1. For research use
2. This product is supplied ready to use. If it is altered by dilution or addition of other components, it should be validated by the user.
3. The reagent is stable until the expiration date shown on the label if it is properly stored. Do not use after the expiration date shown on the label. If the reagents are stored in conditions different from those recommended, such conditions must be validated by the user.
4. Alteration in the appearance of the reagent, such as the precipitation or discoloration indicates instability or deterioration. In such cases, the reagent should not be used.
5. It contains 0.1% sodium azide (CAS-Nr. 26628-22-8) as a preservative, but even so care should be taken to avoid microbial contamination of reagent or incorrect results may occur.
  - Sodium azide (NaN<sub>3</sub>) is harmful if swallowed (R22), if swallowed, seek medical advice immediately and show this container or label (S46).
  - Wear suitable protecting clothing (S36).
  - Contact with acids liberates very toxic gas (R32).
  - Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in metal drains where explosive conditions may develop.
6. All patient specimens and materials with which they come into contact are considered biohazards and should be handled as if capable of transmitting infection<sup>(5)</sup>, and disposed according to the legal precautions established for this type of product. Also recommended is handling of the product with appropriate protective gloves and clothing, and its use by personnel sufficiently

qualified for the procedures described. Avoid contact of samples with skin and mucous membranes. After contact with skin, wash immediately with plenty of water.

7. Use of the reagent with incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.

## **PROCEDURE**

### **Material included**

CD4-APC sufficient for 200 determinations (5 µl MAb to  $10^6$  cells).

### **Material required but not included**

- 633 nm red laser-equipped flow cytometer and appropriate computer hardware and software.
- Test tubes suitable for obtaining samples in the flow cytometer used. Usually tubes with a rounded bottom for 6 mL, 12x 75 mm are used.
- Automatic pipette (100µL) and tips.
- Micropipette with tips.
- Chronometer
- Vortex Mixer
- Isotypic control reagent
- Quicklysis™ lysing solution
- Wash buffer as phosphate buffered saline (PBS) containing 0,1% sodium azide.

### **Preparation**

Whole blood sample must be taken aseptically by means of a venipuncture<sup>(6, 7)</sup> in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). The analysis requires one hundred (100) µl of the whole blood sample per tube, assuming a normal range of approximately  $4$  to  $10 \times 10^3$  leucocytes per µl. For samples with a high white blood cell count, dilute samples with PBS to obtain a concentration of cells approximately equal to  $1 \times 10^4$  cells/µL. Store the blood samples at 18-22°C until they are to be tested. It is advisable to test blood samples within the 24 hours after their extraction. Hemolyzed samples or samples with suspended cell aggregates should be rejected.

1. Mix 100µl of peripheral blood with 5µl of CD4-APC. In the case of working with other body fluids with fewer cells, such as cephalorraquid fluid, bronchoalveolar lavage, gastric lavage, start with an initial volume of 200 µl.  
To evaluate the non-specific binding of the antibody, an appropriated isotype control tube can be prepared.
2. Incubate for 10 minutes at room temperature in the dark.
3. Add 2 ml of Quicklysis™\* erythrocyte lysing solution and incubate the sample for 10 minutes at room temperature in the dark.
4. Acquire directly on the flow cytometer within the first four hours of finishing the sample preparation. If the samples are not acquired immediately after preparation, they should be stored in the dark at 2-8°C. Calibration of the instrument must be done according to the manufacturer's advice. Before acquiring samples, adjust the threshold or discriminator to minimize debris and ensure populations of interest are included. Before acquiring the sample on the flow cytometer, mix the cells on the vortex at low speed to reduce aggregation.

\*Note: The use of other lysing solutions may require the elimination of the lysed red blood cells. Follow the manufacturer's recommended protocol of the lysing solution used.

### **Flow cytometry analysis**

Check that the cytometer is correctly aligned and standardized for light dispersion and fluorescent intensity, and that the right color compensation has been set following the instructions of the cytometer manufacturer.

Visually inspect the CD4 vs SSC dot plot: the helper/inducer T cell population (CD3+CD4+) should appear as a compact CD4+ cluster with low SSC.

The results are commonly reported as a percentage of the total of lymphocyte or leucocytes count present in the sample.

## **LIMITATIONS**

- Blood samples should be stored at 18-22°C and be tested within the 24 hours after they are obtained.
- It is advisable to acquire stained samples on the cytometer as soon as possible to optimize the results. Nonviable cells may stain nonspecifically. Prolonged exposure of whole blood samples to lytic reagents may cause white cell destruction and loss of cells from the target population.
- When using whole blood procedures, all red blood cells may not lyse under following conditions: nucleated red blood cells, abnormal protein concentration or hemoglobinopathies. This may cause falsely decreased results due to unlysed red blood cells being counted as leukocytes.
- Results obtained by flow cytometry may be erroneous if the cytometer laser is misaligned or the gates are improperly set
- Each laboratory should establish a normal range for CD4+ cells using its own test conditions.
- Certain patients may present special problems due to altered or very low number of certain cellular population.
- Cells separated from whole blood by means of density gradients may not have the same relative concentrations of cells as unseparated blood. This may be relatively insignificant for samples from individuals with normal white blood cell counts. In leukopenic patients, the selective loss of specific subsets may affect the accuracy of the determination.
- It is important to understand the normal pattern of expression of this antigen and its relation to the expression of other relevant antigens to carry out an adequate analysis<sup>(8-10)</sup>.
- Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual who may be in an abnormal state of health may show the same leukocyte percentages as a healthy person.

## **EXPECTED VALUES**

Each laboratory should establish its own normal reference ranges for helper/inducer T cells (CD3+CD4+) counts, since such values may be influenced by age, sex and race<sup>(11,12)</sup>. Based on the consulted bibliography and with a merely informative character, the percentage of helper/inducer T cells regarding the total of lymphocytes obtained by CF in healthy individuals is around 33-57% for children of 0-3 years of age, 27-57% for children older than 3 years<sup>(13)</sup> and 28-58% for adults between the ages of 18 and 70<sup>(11)</sup>.







## REFERENCES

1. Orfao A, González de Buitrago JM La citometría de flujo en el laboratorio clínico. Sociedad española de bioquímica clínica y patología Molecular 1995.
2. Stetler-Stevenson M. Flow cytometry analysis of lymphomas and lymphoproliferative disorders. Semin Hematol 2001 Apr;38(2):111-23.
3. Menéndez P, et al. Comparison between a lyse-and-then-wash method and a lyse-non-wash technique for the enumeration of CD34+ hematopoietic progenitor cells. Cytometry (Comm. Clin. Cytometry) 34: 264-271 (1998)
4. GratamaJW, Menéndez P, Kraan J, Orfao A. Loss of CD34+ hematopoietic progenitor cells due to washing can be reduced by the use of fixative-free erythrocyte lysing reagents. J Immunol. Methods 239: 13-23 (2000)
5. Protection of Laboratory Workers from occupationally acquired infections. Second edition; approved guideline (2001). Villanova PA: National Committee for Clinical Laboratory Standards; Document M29-A2.
6. Procedures for the collection of diagnostic blood specimens by venipuncture- approved standard; Fifth edition (2003). Wayne PA: National Committee for Clinical Laboratory Standards; Document H3-A5.
7. Clinical applications of flow cytometry: Quality assurance and immunophenotyping of lymphocytes; approved guideline (1998). Wayne PA: National Committee for Clinical Laboratory Standards; Document H42-A.
8. Lima M et al. TCR $\alpha\beta^+$ /CD4 $^+$  Large Granular Lymphocytosis. A new Clonal T-Cell Lymphoproliferative Disorder. American Journal of Pathology, 163 (2):763-771 (2003)
9. Lima M. et al. Utility of flow cytometry immunophenotyping and DNA ploidy studies for diagnosis and characterization of blood involvement in CD4+ Sézary's syndrome. Haematologica 88(8): 874-887(2003).
10. Gorkczyza W. et al. An approach to diagnosis of T-cell lymphoproliferative disorders by flow cytometry. Cytometry (Clinical cytometry) 50:177-190 (2002)
11. Reichert et al. Lymphocyte subset reference ranges in adult Caucasians. Clin Immunol Immunopathol 60:190-208 (1991)
12. Prince HK et al. Influence of racial background on the distribution of T-cell subsets and Leu-11 positive lymphocytes in healthy blood donors. Diagn Immunol. 3: 33-39 (1985)
13. Kotylo PK et al. Reference ranges for lymphocyte subsets in pediatric patients. Am J Clin Pathol 100:111-5 (1993)

## WARRANTY

This product is warranted only to conform to the quantity and contents stated on the label. There are no warranties that extend beyond the description on the label of the product. Cytognos's sole liability is limited to either replacement of the product or refund of the purchase price.

## EXPLANATION OF SYMBOLS

	Use by (use by YYYY-MM)
	Storage temperature limitation
	Consult instruction for use
<b>RUO</b>	For research use only
	Batch code
	Code number
	Manufacturer

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