



## CD5-PerCP-Cy5.5

Ref: CYT-5C

*For Research Use Only*

### INTENDED USE

CD4-PerCP-Cy5.5 is a monoclonal antibody (MAb) labelled with Peridin chlorophyll protein-Cyanine 5.5 (PerCP-Cy5.5), designed for use as a direct immunofluorescence reagent in the identification and enumeration of cells which express the CD5 antigen by flow cytometry. CD5 is a useful marker for the identification of certain lymphoproliferative syndromes when is used within an extended immunophenotyping panel.

### SUMMARY AND EXPLANATION

The CYT-5C MAb recognizes a 67 kDa antigen expressed by a majority of thymocytes and mature T cells and a subset of B cells <sup>(1-2)</sup>. CD5 is expressed at low density on thymocytes and at high density on all mature T lymphocytes. The majority of T cell malignancies expresses CD5, and almost 85% of T cell acute lymphoblastic leukemias are CD5 positive. Further, CD5 is expressed at low density on a small subset of mature B lymphocytes, which is expanded in several autoimmune disorders as well as in some B-cell derived lymphoproliferative disorders such as chronic lymphocytic leukemia (CLL) and centrocytic leukemia <sup>(1-4)</sup>.

### 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>(5, 6)</sup>.

### REAGENT COMPOSITION

Purified monoclonal CD5 antibody conjugated with conjugated with PerCP-Cy5.5, supplied in phosphate buffered saline with 0,1% sodium azide.

Clone: UCHT2

Isotype: IgG1

Purification: Affinity chromatography

Amount per 0,5 ml vial: 100 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 no 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>(7)</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**

CD5-PerCP-Cy5. sufficient for 100 determinations (5 µl MAb to 10<sup>6</sup> cells).

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

- 488 nm ion argon 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>(8, 9)</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 x 10<sup>3</sup> 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 x 10<sup>4</sup> 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 CD5-PerCP-Cy5.5. In the case of working with other body fluids with fewer cells, such as cerebrospinal 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.

The results are commonly reported as a percentage from 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 CD5+ 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>(1-4)</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 CD5+ cell counts, since leucocyte normal values may be influenced by age, sex and race<sup>(10-12)</sup>. Based on the results obtained in CYTOGNOS and with a merely informative character, the percentage of CD5+ cells regarding the total of lymphocytes obtained by CF in peripheral blood samples of healthy individuals was around 65.01-82.13%.

### **REFERENCES**







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#### 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 (YYYY-MM)
	Storage temperature limitation
	Consult instructions for use
RUO	For research use only
	Batch code
	Catalogue number
	Manufacturer

#### PRODUCED BY

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