CD2-APC-C750

Ref: CYT-2AC750

RUO

For Research Use Only. Not for use in diagnostic procedures

INTENDED USE

CD2-APC-C750 is a monoclonal antibody (mAb) labelled with the tandem allophycocyanine-C750 (APC-C750) designed for use as a direct immunofluorescence reagent in the identification and enumeration of cells which express the CD2 antigen by flow cytometry (FC).

SUMMARY AND EXPLANATION

FC 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. FC 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). CD2 interacts with lymphocyte function-associated antigen (LFA-3) and CD48/BCM1 to mediate adhesion between T-cells and other cell types [(1)].Moreover, CD2 is also implicated in the triggering of T-cells due to its cytoplasmic domain which is implicated in the signaling function [(2)].

CYT-2AC750 mAb recognizes the CD2 antigen a 50 kDa glycoprotein which is present on the human peripheral blood T lymphocytes and NK (78+8% normal peripheral lymphocytes) cells; also expressed by all thymocytes [(3)]. 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 [(4, 5)]

APC-C750 is a tandem dye with a maximun emission peak at 779nm, which grants bright signal, low unspecific noise and high photostability. When excited by light from a red laser, the APC fluorochrome can transfer energy to C750 molecule, which then emits at a longer wavelength. It is recommended to use a 780/60 nm bandpass filter along with a red sensitive detector to use in conjunction antibodies conjugated with APC and APC-C750.

PRINCIPLES OF THE PROCEDURE

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 to 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.[(6, 7).]

The T (CD3+) and NK cells (CD3-CD56+) count is expressed as a percentage of the total amount of lymphocytes or leucocytes present in the sample. Because each flow cytometer has different operating characteristics each laboratory must determine its optimal operating procedure.

REAGENT COMPOSITION

Purified monoclonal CD2 antibody conjugated with the tandem allophycocyanine-C750 (APC-C750), supplied in phosphate buffered saline with ≤0.09% (m/v) sodium azide.
Clone: LT2
Isotype: IgG2b
Amount per 0,15 ml vial: 50 tests (3 µl mAb per determination)
Reagents are not considered sterile.

STORAGE CONDITIONS

The reagent is stable until the expiration date shown on the label, when stored at 2-8º 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 Only. Not for use in diagnostic procedures.
2. This product is supplied ready to use. If it is altered by dilution or addition of other components, such conditions must 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 it 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 0,09% (m/v) sodium azide (CAS-No. 26628-22-8) as a preservative, but even so care should be taken to avoid microbial contamination of reagent or incorrect results may occur.
   Indication(s) of danger:
   H302 Harmful if swallowed
   Safety advice:

P264	Wash thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P301+P312	If swallowed, call a POISON CENTER or doctor/physician if you feel unwell.
P301+P330	If swallowed, rinse mouth.
P501	Dispose of contents/container in accordance with local/regional/national/international regulation.

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 [8], 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

CD2-APC-C750 sufficient for 50 determinations (3 µl mAb per determination).

Material required but not included

- 633 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
- Isotype control reagent
- Quicklysis™ lysing solution (CYT-QL-1)
- Wash buffer as phosphate buffered saline (PBS) containing ≤0.09% (m/v) sodium azide.

Preparation

Whole blood sample must be taken aseptically by means of a venipuncture[9, 10] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). The analysis requires 100 µl of the whole blood sample per tube, assuming a normal range of approximately 4 to 10 x 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 x 10^4 cells/µL. Store the blood samples at 18-22ºC 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.

Suggested procedure:

1. Spin down the vial before each use.
2. Mix 100µl of peripheral blood with 3µl of CD2-APC-C750. 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.
1. To evaluate the non-specific binding of the antibody, an appropriated isotype control tube can be prepared.
2. Incubate for 15 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 to 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 2-8ºC. 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 CD2 vs SSC dot plot: T (CD3+) should appear as a compact CD2+ cluster with low SSC.

LIMITATIONS

- Blood samples should be stored at 18-22ºC and be tested within the 24 hours after they are obtained.
- It is advisable to acquire stained samples to 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 lysed 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 FC may be erroneous if the cytometer laser is misaligned or the gates are improperly set.
- Each laboratory should establish a normal range for T and NK 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. [11-13]
- 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. For this reason, it is advisable to use the test results in combination with other clinical and diagnosis data.

EXPECTED VALUES

Each laboratory should establish its own normal reference ranges for T (CD3+) and NK cells (CD3-CD56+) counts, since such values may be influenced by age, sex and race [14, 15]. Based on the consulted bibliography and with a merely informative character, the percentage of T and NK cells regarding the total of lymphocytes obtained by CF in healthy individuals is around 78+8% and 6-29%[14] respectively.

QUALITY CONTROL

- To obtain optimum results it is advisable to verify the precision of pipettes and that the cytometer is correctly calibrated.
- In multicolor panels fluorochromes emit in different wavelengths but show a certain spectral overlapping which must be corrected by means of electronic compensation. The optimum levels of compensation can be established by analysis in a dot-plot diagram of cells from healthy individuals stained with mutually exclusive monoclonal antibodies conjugated with the fluorochromes to be used in the test. To evaluate the non-specific binding of the antibody, an appropriated isotype control tube can be prepared.
- This product has been manufactured in accordance with standards of production and quality system of the ISO 9001:2008 standard.

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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)
	Catalogue number
	Batch code
	Keep out of sunlight
	Storage temperature limitation
	Consult instructions for use
	Manufacter
	For research use only
	Contains sufficient for <n> tests
	Health hazard/Hazardous to the ozone layer

PRODUCED BY

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