cyCD79a-PE

Ref: CYT-79aPE

For Research Use Only

INTENDED USE

cyCD79a-PE is a monoclonal antibody (MAb) labelled with R-phycoerythrin (PE) designed for use as a direct immunofluorescence reagent in the identification and enumeration of cells which express the CD79α antigen by flow cytometry. CD79α is expressed on B cells at various stages of differentiation, from pre-B cell to plasma cell stage.

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). B lymphocytes are the producers of antibodies and mediate humoral immunity particularly effective against toxins, whole bacteria, and free viruses.

In normal cells CD79α expression are restricted B cell lineage. cyCD79α is found in the cytoplasm of pro B- cells before Ig heavy-chain rearrangement, and is extinguished in terminally differentiated plasma cells. CD79α is expressed in hairy cell leukaemia and in the majority of all low-grade B-cell leukaemias and lymphomas, such as lymphoplasmacytic, follicular, mantle cell, marginal zone and Burkitt's lymphomas. Generally, the CD79α antigen is not found in acute myeloid leukaemia, but the FAB M3 class is a significant exception with more than 50% of cases displaying the antigen in the cytoplasm of malignant cells. Approximately 45% of T- cell ALL cases co-express CD3 and CD79α, and this co-expression phenotype may be clinically relevant [1-4].

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.

CYT-79a-PE should be used with a cell permeabilization kit which gives antibodies access to intracellular structures and leaves the morphological scatter characteristics of cells intact.

REAGENT COMPOSITION

Purified monoclonal cyCD79a Antibody conjugated with R-phycoerythrin (PE), supplied in phosphate buffered saline with 0,1% sodium azide.

Clone: HM57.

Isotype: IgG1.

Amount per 1 ml vial: 100 tests (10 µl/ test).

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.
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 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_3) 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 [5], 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

Anti Human cyCD79a-PE Antibody sufficient for 100 determinations (10 µl/ test).

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
- Cell permeabilization kit
- 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 [6, 7] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). The analysis requires 50 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.

1. Transfer 50 µl of the cell suspension to be analyzed (whole blood, bone marrow or mononuclear cells) to a test tube.
2. In case of use a staining protocol of cell surface antigens and intracellular antigens, add the appropriate volume of antibodies to surface staining. Mix gently by using a vortex mixer and incubate at room temperature for 15 minutes.
3. Add 100 µl of Fixation Reagent. Mix gently to ensure that the cells are in suspension.
4. Incubate at room temperature for 15 minutes.
5. Add 2 ml PBS and mix gently by using a vortex mixer.
6. Centrifuge at 540g for 5 minutes.
7. Aspirate the supernatant, leaving approximately 50 µl of fluid.
8. Mix thoroughly by using a vortex mixer to ensure that the cells are in suspension and add 100 µL of Permebilization Reagent. Add 10 µL of CYT-79aPE. Mix gently
   Use a non-reactive monoclonal antibody of the same isotype, and conjugated with the same fluorochrome, as a negative control.
9. Incubate in the dark at room temperature for 15 minutes.
10. Add 2 ml PBS and mix gently by using a vortex mixer.
11. Centrifuge at 540g for 5 minutes.
12. Aspirate the supernatant, leaving approximately 50 µl of fluid.
13. Resuspend the cell pellet in 300 µl PBS.
14. Acquire on a flow cytometer within the first three hours of finishing the sample preparation.

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 of lymphocyte or leucocytes count present in the sample.

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 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 B 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 [8].
- 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 B cells counts, since leucocyte normal values may be influenced by age, sex and race [9].

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.

REFERENCES

1. Deegan MJ, B Lymphocytes and plasma cells: their development and identification. In: Keren DF, editor. Flow cytometry in clinical diagnosis. Chicago: ASCP Press; p.1 139-163 (1989).
2. Braylan RC, Orfao A, Borowitz MJ, Davis BH. Optimal number of reagents required to evaluate hematolymphoid neoplasias: results of an international consensus meeting. Cytometry 46: 23-7 (2001)
3. Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood 90(8): 2863-2892 (1997)
4. Stetler-Stevenson M. Flow cytometry analysis of lymphomas and lymphoproliferative disorders. Semin Hematol 2001 Apr; 38(2):111-23.
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. Loken MR, Wells DA. Normal antigen expression in Hematopoiesis: basis for interpreting leukemia phenotypes. In Immunophenotyping. Wiley-Liss (2000).
9. Reichert et al. Lymphocyte subset reference ranges in adult Caucasians. Clin Immunol Immunopathol 60:190-208 (1991)

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
	Manufacter

PRODUCED BY

CYTOGNOS SL
Polígono La Serna, Nave 9
37900 Santa Marta de Tormes
Salamanca (España)
Phone: + 34-923-125067
Fax: + 34-923-125128
Ordering information: admin@cytognos.com
Technical information: support@cytognos.com

www.cytognos.com