CD45-Pacific Blue™

Ref: CYT-45PBZ

For Research Use Only

INTENDED USE

CD45-Pacific Blue™ is a monoclonal antibody (MAb) labelled with Pacific Blue™ designed for use as a direct immunofluorescence reagent in the identification and enumeration of cells which express the CD45 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.

Leukocytes are named according to structure as being either granulocytes or agranulocytes, and according to the function as either phagocytes or immunocyte. The granulocytes, which include neutrophils, basophils and eosinophils, are all phagocytes. Of the agranulocytes, the monocytes and macrophages are phagocytes whereas the lymphocytes are immunocytes. The granulocytes have granules in their cytoplasm, which contain enzymes which are capable of killing microorganisms and destroying debris ingested by the process of phagocytosis.

The CYT-45PBZ MAb reacts with the leucocyte common antigen (LCA) complex, which is present on lymphocytes, monocytes, granulocytes, eosinophils, basophils and their progenitors. This reagent can be used in the characterization studies for immunophenotyping of leucocytes, which are widely applied in the characterization and follow-up of immunodeficiencies, autoimmune diseases, leukemias, etc [1, 2]

Pacific Blue™ is excited with the violet laser (405nm) and emits at 550nm. This fluorochrome provides maximum resolution and narrow emission peaks, which results in little spectral overlap and minimal compensation requirements. In multicolor panel it is recommended the use of this fluorochrome combined with Orange Cytognos 515, which is also excited with the violet laser and emits at 515nm.

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 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.[3, 4].

This MAb is a single-color direct immunofluorescence reagent for establishing an optimal leucocyte gate for immunophenotying of erythrocyte-lysed whole blood. Because each flow cytometer has different operating characteristics each laboratory must determine its optimal operating procedure.

REAGENT COMPOSITION

Purified monoclonal CD45 antibody conjugated with Pacific Blue™, supplied in phosphate buffered saline with 0,1% sodium azide.
Clone: GA90.
Isotype: IgG2a.
Amount per 0,5 ml vial: 100 tests (5 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.
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 CD45-Pacific Blue™ antibody can be used for 100 determinations (5 µl MAb per determination).

Material required but not included

- 405 nm violet 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
- 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 one hundred (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.

1. Mix 100µl of peripheral blood with 5µl of CD45-Pacific Blue™. 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 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 CD45 vs SSC dot plot: lymphocytes stained exhibit low SSC with high CD45 antigen expression, monocytes stained show intermediate SSC with high CD45 antigen expression and neutrophils and eosinophils stained exhibit high SSC with intermediate CD45 antigen expression.
The results are used for establishing an optimal leucocyte gate for immunophenotying of erythrocyte-lysed whole blood.

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 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 leucocytes 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, 9].
- 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 CD45+ cells counts, since leucocyte normal values may be influenced by age, sex and race [10, 11].

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.

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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
	Manufacter

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