CD52-PE

Ref: CYT-52PE

For Research Use Only

INTENDED USE

CD52-PE is a monoclonal antibody (MAb) labelled with R-phycoerythrin (PE) designed for flow cytometry use as a direct immunofluorescence reagent in the identification and enumeration of CD52 antigen-expressing cells.

SUMMARY AND EXPLANATION

Flow Cytometry (FC) is a powerful tool in analytical and quantitative characterization of cells which provides rapid and multiparametric analysis of heterogeneous cell populations on a cell-by-cell basis. Flow cytometry is performed on cell suspension after incubating it with fluorescent-labelled antibodies directed against specific cellular proteins. Positive cells relative fluorescence intensity indicates the amount of antibody bonded to specific cell sites providing information about antigen expression [(1)].

Several antibodies have found clinical application due to its functions in the immune system. CD52, also known as CAMPATH-1, is a 21 kDa highly glycosylated GPI-anchored which is present at high levels on lymphocytes, monocytes/macrophages, eosinophils, erythroid cells and epithelial cells of male reproductive tract. All of the functions of CD52 are still not defined but it is known to play a role in T-cell and complement activation [(2, 3)].

CD52 has been used as a depletion target for relapsed/refractory chronic lymphocytic leukaemia (CLL)/lymphoma and immunosuppression since it is a potent target for complement-mediated lysis, antibody-mediated cellular cytotoxicity and other therapies as stem cell transplantation. CYT-52PE MAb can be used in several studies to determine its complete functions and other applications are being evaluated including T cell lymphoma (PTCL), T cell pro-lymphocytic leukaemia, and cutaneous T cell lymphoma [(4, 5)].

PROCEDURE PRINCIPLES

Flow cytometry is an innovative technology that can evaluate simultaneously different characteristics for a single cell. Flow cytometers use hydrodynamic focusing to individually present cells to one or more laser beams. As cells are intercepted by light a set detectors recover signals of two different kinds: those generated by dispersed light (FSC/SSC), which mainly reflect cell size and internal complexity, and those related to fluorochromes light emission when cells are labelled. Recovered signals are then amplified by a series of linear and logarithmic amplifiers and converted in electrical signals large enough to be plotted graphically.

Fluorochrome-labelled monoclonal antibodies bind specific antigens, therefore cell populations carrying this antigen will be detected when the reagent is added to a sample and passed through a flow cytometer.

Detection of targeted populations can be hindered by erythrocytes presence. This problem can be avoided by their removal using a red blood cell lysing solution previous to sample acquisition. Quicklysis™ (CYT-QL-1) erythrocyte lysing solution without fixatives is recommended since it requires no washing steps. Quicklysis™ use minimises sample handling and avoids centrifuge processed which are usually associated to cell losses [(6, 7)].

CD52 positive cells are expressed as a percentage of total lymphocytes or leucocytes present in a sample. Since every flow cytometer has different operating characteristics each laboratory must determine its optimal operating procedure.

REAGENT COMPOSITION

The purified monoclonal CD52 antibody conjugated with R-phycoerythrin (PE) is supplied in phosphate-buffered saline (PBS) containing 0.1% sodium azide.

Clone: YTH34.5.

Isotype: IgG2b.

Amount per 1 ml vial: 200 tests (5 µl MAb per determination).

Reagent is considered non-sterile.

STORAGE CONDITIONS

The reagent is stable when stored at 2-8 ºC until expiration date shown on label. The reagent should not be frozen or exposed to direct light during storage or during cell incubation. Reagent vial should be kept dry and once open stored in vertical position to avoid any possible spillage.

WARNINGS AND RECOMMENDATIONS

1. For research use only.
2. This product is supplied ready to use, any modification by dilution or addition of other compounds should be validated by the user.
3. The reagent is stable until its expiry date when properly stored. Do not use it after expiration date shown on label. If product is stored in conditions different from those recommended, such conditions must be validated by the user.
4. Alteration in reagent appearance, such as 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 care should anyway be taken to avoid microbial contamination of reagent and reduce incorrect results that can probably arise from that contamination.
    - 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).
    - On disposal, flush with large amounts of water to prevent azide build-up in metal plumbing since explosive conditions may develop.
6. All patient specimens and materials are considered biohazards and should be handled as if capable of transmitting infection [(8)]. Disposal should be made according to the established legal precautions. It is also recommendable the use of appropriate protective gloves and clothing when handling this product. Product use should be made by personnel qualified to perform the described procedures. Avoid sample contact with skin or mucous membranes. Wash immediately with abundant water if skin contact has occurred.

7. Reagent use with incubation times or temperatures different from those recommended may cause erroneous results. Any changes in procedure must be validated by the user.

PROCEDURE

Material included

CD52-PE can be used for 200 determinations (5 µl MAb per determination).

Material required but not included

- 488 nm ion argon laser-equipped flow cytometer and appropriate computer hardware and software.
- Test tubes suitable for the used flow cytometer. Usually 6 mL tubes (12x 75 mm) with a rounded bottom are used.
- Automatic pipette (100µL) and tips.
- Micropipette and tips.
- Chronometer.
- Vortex Mixer.
- Isotypic control reagent.
- Quicklysis™ lysing solution.
- Wash buffer (phosphate buffered saline (PBS) containing 0.1% sodium azide).

Preparation

Whole blood sample must be taken aseptically by venipuncture [9, 10] in a sterilized tube containing an appropriate anticoagulant (use of EDTA is recommended). Flow cytometry analysis require one hundred (100) µL whole blood sample per tube, assuming a normal range of approximately 4 to 10 x 10^3 leucocytes per µL. Samples with high white blood cell count should be diluted with PBS to obtain an approximate cell concentration of 1 x 10^4 cells/µL. Store blood samples at 18-22ºC until they are to be tested. It is advisable to test blood samples within 24 hours after their extraction. Samples with suspended cell aggregates or haemolysed should be rejected.

1. Mix 100µL of peripheral blood with 5µL of CD52-PE. In case of working with other body fluids with fewer cells, such as cerebrospinal fluid, bronchoalveolar lavage, gastric lavage, etc., start with an initial volume of 200 µL.
   To evaluate non-specific binding of the antibody, an appropriated isotype control tube can be prepared.
2. Incubate in the dark at room temperature for 10 minutes.
3. Add 2 mL of Quicklysis™* erythrocyte lysing solution and incubate sample in the dark at room temperature for 10 minutes.
4. Acquisition in the flow cytometer should be performed within the first four hours after sample preparation. If samples are not acquired immediately after preparation, they should be stored in the dark at 2-8ºC. Instrument calibration must be done according to manufacturers' advice. Before acquiring samples, adjust threshold or discriminator to minimise debris and ensure that populations of interest are included. Also, previous to acquisition samples should be mixed on a vortex (low speed) to reduce cell aggregation.

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

Flow cytometry analysis

Confirm that the cytometer is correctly aligned and standardised for light dispersion and fluorescent intensity. Compensation should be set following cytometer manufacturer instructions.
Display in a plot CD52 with an appropriate parameter (e.g. CD3 for T-cells) in order to identify interest population.
Results are commonly reported as a percentage of sample total lymphocyte or leucocyte count present.

LIMITATIONS

- Blood samples should be stored at 18-22ºC and tested within 24 hours after they were obtained.
- It is advisable to acquire stained samples as soon as possible to optimise results. Non-viable cells may show unspecific staining. Prolonged exposure of whole blood samples to lytic reagents may cause white cell destruction and targeted population cell loss.
- When using whole blood procedures some red blood cells may not lyse, for instance if there are nucleated red blood cells or if abnormal protein concentration and haemoglobinopathies are observed. This may cause misleading results since unlysed red blood cells are counted as leucocytes.
- Results obtained by flow cytometry may be erroneous if cytometer laser is misaligned or if gates are incorrectly set.
- Each laboratory should establish a normal range for CD52+ cells using its own test conditions.
- Certain patients may present special problems due to altered or very low number of a certain cellular population.
- Cells separated from whole blood by means of density gradients may not have the same relative concentration as in whole blood. This may be relatively insignificant in individuals with normal white blood cell counts. In leucopenic patients, the selective loss of specific subsets may affect determination accuracy.
- Knowledge of antigen normal expression pattern and its relation to other relevant antigens is paramount to carry out an adequate analysis [2, 11].
- Abnormal states of health are not always represented by abnormal percentages of certain leucocyte populations. An individual in an abnormal state of health may show the same leucocyte percentage as a healthy person.

EXPECTED VALUES

Each laboratory should establish their own normal reference ranges for CD52 positive cells since those values may be influenced by age, sex and race [12-13]. CD52 antigen is exceptionally abundant found at a density of up to 5x10^5 binding sites/cell on more than 95% peripheral blood lymphocytes, tonsillar cells, thymocytes, and monocytes [14]. This information is based on the consulted bibliography and has mere informative character.

QUALITY CONTROL

- Pipettes precision and cytometer calibration should be verified to obtain optimal results.
- In multicolour panels, fluorochromes emit in wavelengths that can show certain spectral overlap which must be corrected by electronic compensation. Optimal compensation levels can be established by analysing cells from healthy individuals stained with mutually exclusive monoclonal antibodies conjugated with appropriate fluorochromes.
- Non-specific binding of the antibody can be evaluated using an appropriated isotype control tube.

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WARRANTY

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

EXPLANATION OF SYMBOLS

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

PRODUCED BY

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