CD3-FITC / CD16-PE
Ref: CYT-3F8-16PE7

For research use only

INTENDED USE
CD3-FITC/CD16-PE is a two-color direct immunofluorescence reagent for use in flow cytometry designed for the identification and enumeration of T Lymphocytes (CD3+) and Natural Killer (CD3-CD16+) cells.

SUMMARY AND EXPLANATION
Flow Cytometry (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. 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). T lymphocytes (CD3+), the precursors of which originate in the bone marrow and then migrate and mature in the thymus, can be subdivided as well in functionally different populations. The most clearly defined of these are helper/inducer T cells (CD3+CD4+) and suppressor/cytotoxic T cells (CD3+CD8+). T cells are the mediators of cell immunity. NK cells (CD3-CD16+) mediate cytotoxicity against certain tumors and virus-infected cells. NK-mediated cytotoxicity does not require class I or class II major histocompatibility complex (MHC) molecules to be present on the target cell.
The CD3-FITC/CD16-PE reagent recognizes the antigens CD3, present in T cells, and CD16, present in NK cells. This reagent can be used in the characterization studies for immunophenotyping of lymphocytes. These studies are widely applied in the characterization and follow-up of acute and chronic lymphoproliferative disorders, immunodeficiencies, autoimmune diseases, etc [1-3]. The CD16 antigen is also detected on macrophages and neutrophils.

PRINCIPLES OF THE PROCEDURE
Flow cytometry 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 monoclonal antibodies (MAb) present in the reagent (CD3-FITC and CD16-PE) bind specifically to the antigens they are directed against, allowing the detection by FC of the cell populations carried by the antigens.
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.[4, 5].
The T cells (CD3+) and NK cells (CD3-CD16+) count is expressed as a percentage of the total amount of lymphocytes or leucocytes present in the sample which can itself be determined by FC based on its typical pattern of FSC/SSC (size/granularity or complexity). Because each flow cytometer has different operating characteristics each laboratory must determine its optimal operating procedure.

REAGENT COMPOSITION
CD3-FITC/CD16-PE reagent is provided in phosphate buffered saline with 0.1% sodium azide. It contains fluorescein isothiocyanate (FITC)-labeled CD3, clone Cris-7; isotype IgG2a and phycoerythrin (PE)-labeled CD16, clone 3G8, isotype IgG1.
Purification: Affinity chromatography
Amount per 1 ml vial: 100 tests (10 µl reagent to 10^6 cells)
Reagents are not considered sterile.

STORAGE CONDITIONS
The reagent is stable until the expiration date shown on the label, when stored at 2-8ºC. 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 not 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 [6], 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

CD3-FITC/CD16-PE sufficient for 100 determinations (10 µl reagent to 10^6 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[7, 8] 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 10µl of CD3-FITC/CD16-PE. 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 reagent, 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 2-8ºC. 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 (FSC/SSC on linear scale) and fluorescent intensity (FL1, FL2, FL3 and FL4 on logarithmic scale) 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 the total 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 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. [3, 9-11]
- 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 lymphocyte (CD3+) and NK cell (CD3-CD16+) counts, since such values may be influenced by age, sex and race [12-14]. Based on the consulted bibliography and with a merely informative

character, the percentage of CD3+ cells regarding the total of lymphocytes obtained by CF in healthy individuals is around 55-82% for children of 0-3 years of age[14] and 61-85% for adults between the ages of 18 and 70 [12]. The percentage of NK cells regarding the total of lymphocytes obtained by CF in healthy individuals is around 6-29% for adults between the ages of 18 and 70[12].

REFERENCES

1. Orfao A, González de Buitrago JM La citometría de flujo en el laboratorio clínico. Sociedad española de bioquímica clínica y patología Molecular 1995.
2. Stetler-Stevenson M. Flow cytometry analysis of lymphomas and lymphoproliferative disorders. Semin Hematol 2001 Apr;38(2):111-23.
3. Okuno SH, Tefferi A, Hanson C, Katzmann JA, Li CY, Witzig TE. Spectrum of diseases associated with increased proportions or absolute numbers of peripheral blood natural killer cells. BrJ Haematol. 93: 810-812 (1996)
4. Menéndez P, et al. Comparison between a lyse-and-then-wash method and a lyse-non-wash technique for the enumeration of CD34+ hematopoietic progenitor cells. Cytometry (Comm. Clin. Cytometry) 34: 264-271 (1998)
5. GratamaJW, Menéndez P, Kraan J, Orfao A. Loss of CD34+ hematopoietic progenitor cells due to washing can be reduced by the use of fixative-free erytrocyte lysing reagents. J Immunol. Methods 239: 13-23 (2000)
6. Protection of Laboratory Workers from occupationally acquired infections. Second edition; approved guideline (2001). Villanova PA: National Committee for Clinical Laboratory Standards; Document M29-A2.
7. 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.
8. 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.
9. Gorczyza W. et al. An approach to diagnosis of T-cell lymphoprolipherative disorders by flow cytometry. Cytometry (Clinical cytometry) 50:177-190 (2002)
10. Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood 90(8): 2863-2892 (1997)
11. Loken MR, Wells DA. Normal antigen expression in Hematopoiesis: basis for interpreting leukemia phenotypes. In Immunophenotyping. Wiley-Liss (2000)
12. Reichert et al. Lymphocyte subset reference ranges in adult Caucasians. Clin Immunol Immunopathol 60:190-208 (1991)
13. Prince HK et al. Influence of racial background on the distribution of T-cell subsets and Leu-11 positive lymphocytes in healthy blood donors. Diagn Immunol. 3: 33-39 (1985)
14. Kotylo PK et al. Reference ranges for lymphocyte subsets in pediatric patients. Am J Clin Pathol 100:111-5 (1993)

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