EXCLUSION KIT FOR DENDRITIC CELLS STUDIES

CD3FITC+CD19FITC+CD56FITC+CD14FITC

Ref: CYT-DENDF

For Research Use Only

INTENDED USE

CYT-DENDF is a combination of monoclonal antibodies directed against T-lymphocytes (CD3), B-Lymphocytes (CD19), natural killer (CD56) and monocytes (CD14) all conjugated with FITC. Peripheral blood dendritic cells can be distinguished from other leucocytes by their characteristic lack of staining with this combination [(1)].

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. 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.

Dendritic cells constitute a heterogeneous group of cells which play a crucial role in the immune system since they represent the most potent professional antigen-presenting cells for the initiation of immune responses. This is related to their widespread localization in all sites of antigen entry, their high expression of immunomodulatory molecules necessary for T cell activation, and their production of cytokines.

In recent years there has been an increasingly high interest on the study of dendritic cells, particularly due to their possible application in immunotherapy. Accordingly, the ability of dendritic cells to stimulate primary T lymphocyte and T cell-dependent immune responses may provide opportunities for therapeutic intervention in bone marrow and solid organ transplantation, as well as in autoinmune diseases. In addition, protocols for clinical immunotherapy programmes, targeted on malignant cell antigens or infectious agents, have been designed to exploit dendritic cells as a natural adjuvant for optimal therapeutic vaccination.

Dendritic cells can be identified in human peripheral blood as the fraction of nucleated cells which do not show reactivity for CD3, CD19, CD56 and CD14 antigens and which at the same time are positive for HLA-DR. At flow cytometry these cells display a typical light scatter pattern, with FSC/SSC intermediate values between lymphocytes and monocytes.

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-labeled antibodies present 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 an erythrocyte lysing solution containing fixatives previous to acquire the sample on the cytometer.

REAGENT COMPOSITION

CYT-DENDF is provided in phosphate buffered saline with ≤0.09% (m/v) sodium azide. It contains:
CD3FITC, Clone: Cris7 (IgG2a)
CD19FITC, Clone: HD37 (IgG1)
CD56FITC, Clone: C5.9 (IgG2b)
CD14FITC, Clone: 18D11 (IgG2a)
Amount per 1 mL vial: 50 tests (20 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 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 0,09% (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 [2], 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

CD3FITC+CD19FITC+CD56FITC+CD14FITC, sufficient for 50 determinations (20 µL/test).

Material required but not included

- 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.
- Centrifuge.
- Pasteur pipette or vacuum system.
- Isotype control reagent.
- Erythrocyte lysing solution.
- Wash buffer as phosphate buffered saline (PBS) + ≤0.09% (m/v) sodium azide.

Preparation

Whole blood sample must be taken aseptically by means of a venipuncture [3, 4] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). 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. Add 20 µl of reagent per 100 µl of whole blood.
2. Mix gently
3. Incubate for 15 min at room temperature (RT) protected from light.
4. Add 2 mL of an erythrocyte lysing solution containing fixatives.
5. Mix well and cover the tubes with parafilm.
6. Incubate for 10 min at room temperature (RT) protected from light. It is recommended maintain the tubes in horizontal and shake from time to time during incubation time.
7. Read in a flow cytometer in the following three hours after their preparation. Where the samples are not going to be read immediately after their preparation, it is recommended maintain the samples at 4º C in the dark until their processed.

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. Non-viable cells may stain non-specifically. 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 FC 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 bearing Kappa and Lambda Light Chains 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.
- 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.

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. Almeida J, Bueno C, Alguero MC, Sanchez ML, Cañizo MC, Fernandez ME, Vaquero JM, Laso FJ, Escribano L, San Miguel JF, Orfao A. Extensive characterization of the immunophenotype and pattern of cytokine production by distinct subpopulations of normal human peripheral blood MHC II+/lineage- cells. Clin Exp Immunol. 1999 Dec;118(3):392-401.
2. Protection of Laboratory Workers from occupationally acquired infections. Second edition; approved guideline (2001). Villanova PA: National Committee for Clinical Laboratory Standards; Document M29-A2.
3. 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.
4. 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.

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' 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
	Keep out of sunlight
	Consult instructions for use
	For research use only
	Batch code
	Catalogue number
	Manufacter

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