IgG2b-FITC/IgG2b-PE/IgG1-PECy5 Mouse isotypic control
Ref: CYT-IC7F7PE5C

CE

For In Vitro Diagnostic use

INTENDED USE
CYT-IC7F7PE5C is used as quality control reagent in flow cytometry to monitor the level of non-specific antibody binding in cell surface staining procedures which use monoclonal antibodies of the mouse IgG2b subclass conjugated with fluorescein isothiocyanate (FITC), monoclonal antibodies of the mouse IgG2b subclass conjugated with phycoerythrin (PE) and monoclonal antibodies of the mouse IgG1 subclass conjugated with phycoerythrin-cyanine 5 (PECy5).

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.
CYT-IC7F7PE5C is a three-color direct immunofluorescence reagent intended for use as a negative control in flow cytometry. Its use enables an estimation of non-specific binding of FITC, PE and PECy5 conjugated mouse monoclonal antibodies to cell surface components in peripheral blood samples.

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) bind specifically to the antigens they are directed against, allowing the detection by FC of the cell populations carried by the antigen. As negative control, an appropriated isotype control tube can be prepared to evaluate the non-specific binding of the MAb.

REAGENT COMPOSITION
CYT-IC7F7PE5C is provided in in phosphate buffered saline with 0,1% sodium azide. It contains the following mixture of negative controls:

- Mouse IgG2b negative control, FITC conjugate, clone: MCG2b.
- Mouse IgG2b negative control, PE conjugate, clone: MCG2b.
- Mouse IgG1 negative control, PECy5 conjugate, clone: ZX3

Purification: Purified from mouse ascetic fluid by affinity chromatography.
Amount per 1,5 ml vial: 100 tests (15 µl negative control 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 2-8º 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 in vitro diagnostic use.
2. This product is supplied ready to use. If it is altered by dilution or addition of other components, it will be invalidated for in vitro diagnostic use.
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 [(1)], 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

Mouse IgG2b-FITC/IgG2b-PE/IgG1-PECy5 isotypic control sufficient for 100 determinations (15 µl negative control 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
- Quicklysis™ lysing solution

Preparation

Whole blood sample must be taken aseptically by means of a venipuncture[2, 3] 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.

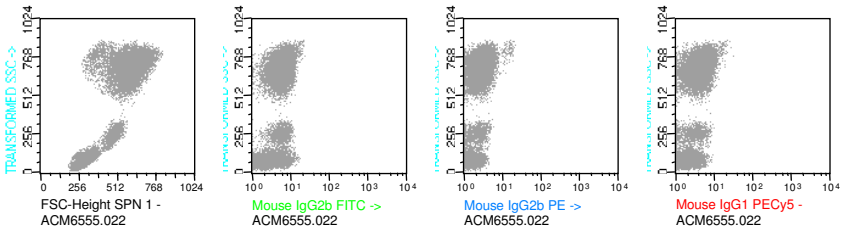
1. Mix 100µl of peripheral blood with 15µl of the mouse IgG2b-FITC/IgG2b-PE/IgG1-PECy5 isotypic control. In the case of working with other body fluids with fewer cells, such as cephalorraquid fluid, bronchoalveolar lavage, gastric lavage, start with an initial volume of 200 µl.
2. Incubate for 10 minutes 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 at 2-8ºC in the dark. 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 mouse IgG2b-FITC/IgG2b-PE/IgG1-PECy5 isotypic control is used as negative control for immunofluorescence staining with antibodies of the mouse IgG2b subclass conjugated with FITC, the mouse IgG2b subclass conjugated with PE, and the mouse IgG1 subclass conjugated with PECy5. The following figure shows representative flow cytometry data on peripheral blood (healthy individual) stained with the reagent.



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

QUALITY CONTROL

- To obtain optimum results it is advisable to verify the precision of pipettes and that the cytometer is correctly calibrated.
- The fluorochromes fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), R-phycoerythrin-cyanine 5 (PECy5), allophycocyanin (APC) emit in different wavelengths but show a certain spectral overlapping which must be corrected by means of electronic compensation if combinations of different monoclonal antibodies are used conjugated with these fluorochromes. 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.

REFERENCES

1. Protection of Laboratory Workers from occupationally acquired infections. Second edition; approved guideline (2001). Villanova PA: National Committee for Clinical Laboratory Standards; Document M29-A2.
2. 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.
3. 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'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
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	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

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