

# IOTest® CD69-PC5

REF IM2656  
100 tests; 1 mL  
10 µL / test



IOTest  
Conjugated Antibody



| ENGLISH        | Specifications  |
|----------------|---|
| Specificity    | CD69  |
| Clone          | TP1.55.3  |
| Hybridoma      | P3-X63-Ag.8.653 x Balb/c                                |
| Immunogen      | Human PBL activated for 24h with PMA and CD3 mAb        |
| Immunoglobulin | IgG2b   |
| Species        | Mouse   |
| Source         | Ascites   |
| Purification   | Protein A affinity chromatography                       |
| Fluorochrome   | Phycoerythrin cyanin 5.1 (PC5)                          |
| λ excitation   | 488 nm  |
| Emission peak  | 670 nm  |
| Buffer         | PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN <sub>3</sub> |

## USE

This fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the CD69 antigen present in human biological samples using flow cytometry.

## PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes.

Specific staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The red cells are then removed by lysis and the leucocytes, which are unaffected by this process, are analyzed by flow cytometry.

The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow-angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the so delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

## EXAMPLES OF CLINICAL APPLICATIONS

The CD69 is a very early activation antigen that is rapidly up regulated on the surface of leucocytes and platelets upon activation. Since CD69 seems to be involved in the pathogenesis of some inflammatory diseases, this reagent could be used to characterize and enumerate CD69<sup>+</sup> activated cell populations in immune system disorders : viral infections (1), rheumatoid arthritis (2), poorer IVF treatment and pregnancy outcome (3), non atopic asthma (4), defect in lymphocytes proliferation or activation (5). On the other hand, it enables the follow-up and functions monitoring of lymphocytes during organ transplantation (6) or specific immunotherapy (7).

## STORAGE AND STABILITY

The conjugated liquid forms must be kept at between 2 and 8°C and protected from light, before and after the vial has been opened.

Stability of closed vial: see expiry date on vial.

Stability of open vial: the reagent is stable for 90 days.

## PRECAUTIONS

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.

3. Let it come to room temperature (18 – 25°C) before use.
4. Minimize exposure to light.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. Antibody solutions containing sodium azide (NaN<sub>3</sub>) should be handled with care. Do not ingest and avoid contact with the skin, mucosa and eyes.  
Moreover, in an acid medium, sodium azide can form the potentially dangerous hydrazoic acid. If it needs to be disposed of, it is recommended that the reagent be diluted in a large volume of water before pouring it into the drainage system so as to avoid the accumulation of sodium azide in metal pipes and to prevent the risk of explosion.
7. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
8. Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.
9. Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

## SAMPLES

Venous blood or bone marrow samples must be taken using sterile tubes containing an EDTA salt as the anticoagulant. The use of other anticoagulants is not recommended.

The samples should be kept at room temperature (18 – 25°C) and not shaken. The sample should be homogenized by gentle agitation prior to taking the test sample.

The samples must be analyzed within 24 hours of venipuncture.

## METHODOLOGY

### NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 10, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads. For example: Flow-Set™ Fluorospheres (Ref. 6607007).
- Red cell lysis reagent with washing stage after lysis. For example: VersaLyse™ (Ref. A09777).
- Leucocyte fixation reagent. For example: IOTest 3 Fixative Solution (Ref. A07800).
- Isotypic control: Mouse IgG2b-PC5.
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

## PROCEDURE

**NOTE:** The procedure below is valid for standard applications. Sample and/or VersaLyse volumes

for certain Beckman Coulter applications may be different. If such is the case, follow the instructions on the application's technical leaflet. For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed in the presence of the isotypic control.

1. Add 10 µL of specific IOTest conjugated antibody to each test tube, and the necessary amount of the isotypic control to each control tube.
2. Add 100 µL of the test sample to both tubes. Vortex the tubes gently.
3. Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4. Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used. As an example, if you wish to use VersaLyse (Ref. A09777), refer to the leaflet and follow preferably the procedure called "with concomitant fixation", which consists of adding 1 mL of the "Fix-and-Lyse" mixture prepared extemporaneously. Vortex immediately for one second and incubate for 10 minutes at room temperature, protected from light.

If the sample does not contain red cells, add 2 mL of PBS.

5. Centrifuge for 5 minutes at 150 x g at room temperature.
6. Remove the supernatant by aspiration.
7. Resuspend the cell pellet using 3 mL of PBS.
8. Repeat step 5.
9. Remove the supernatant by aspiration and resuspend the cell pellet using:
  - 0.5 mL or 1 mL of PBS plus 0.1% of formaldehyde if the preparations are to be kept for more than 2 hours and less than 24 hours. (A 0.1% formaldehyde PBS can be obtained by diluting 12.5 µL of the IOTest 3 Fixative Solution (Ref. A07800) at its 10X concentration in 1 mL of PBS).
  - 0.5 mL or 1 mL of PBS without formaldehyde, if the preparations are to be analyzed within 2 hours.

**NOTE:** In all cases, keep the preparations between 2 and 8°C and protected from light.

## PERFORMANCE

### SPECIFICITY

The CD69 molecule, also designated as Activation Inducer Molecule (AIM), is a phosphorylated disulfide-linked 27-33 kDa homodimer composed of differentially glycosylated subunits.

CD69 is a type II integral membrane protein with an extracellular C-type lectin domain (8).

It is the earliest inducible cell surface glycoprotein to appear upon «in vitro» activation of T, NK, and B cells.

CD69 is involved in signal transduction during the initial steps of cell activation.

The majority of peripheral blood NK cells are negative for CD69, but they express AIM shortly after activation with PMA, IL-2, alpha interferon, CD16 monoclonal antibody (10).

CD69 is constitutively expressed in a sub-population of thymocytes and platelets (11). In NK cells and platelets CD69 acts as a triggering molecule.

TP1.55.3 reacts with activated T lymphocytes but has weak or no effect on the induction of T-cell proliferation and IL-2 production, respectively (9).

TP1.55.3 immunoprecipitates the 60 kDa homodimer and both 27 kDa and 33 kDa subunits from PBL activated (with PMA and anti-CD3 mAbs) under reduced and nonreduced conditions, respectively (9).

### LINEARITY

To test the linearity of staining of this reagent, a positive cell line (activated PBL) and a negative cell line (PBL) were mixed in different proportions with a constant final number of cells, so that the positive / negative cell line ratio of the mixture ranged from 0 to 100%.

Aliquots were stained using the procedure described above and linear regression between the expected values and the observed values was calculated.

| Specificity | Linear regression     | Linearity (R <sup>2</sup> ) |
|-------------|-----------------------|-----------------------------|
| CD69        | Y = 0.9873 X + 0.3794 | 0.9994                      |

### EXPECTED VALUES

Each laboratory must compile a list of reference values based upon a group of healthy donors from the local population. This must be done by taking age, sex and ethnic group into account, as well as any other potential regional differences.

In our laboratories, the whole blood samples of 10 healthy adults were used. The results obtained are given in the table below:

| Target population              | Number | Mean (%)* | SD    | CV (%) |
|--------------------------------|--------|-----------|-------|--------|
| CD69 <sup>+</sup> Lymphocytes  | 10     | 27.45     | 15.61 | 56.87  |
| CD69 <sup>+</sup> Monocytes    | 10     | 18.66     | 23.72 | 127.10 |
| CD69 <sup>+</sup> Granulocytes | 10     | 22.91     | 20.21 | 88.24  |

(\* Mean of % of the stained events among the target population / isotypic control)

### INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the positivity of a sample containing positive cells (activated PBL from peripheral blood from the same donor) were carried out. The results obtained are summarized in the following table:

| Lymphocytes       | Number | Mean (%) | SD   | CV (%) |
|-------------------|--------|----------|------|--------|
| CD69 <sup>+</sup> | 12     | 98.94    | 0.15 | 0.15   |

### INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same sample containing positive cells (activated PBL from peripheral blood from the same donor), 12 measurements of the positivity were carried out by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following table:

Cytometer n° 1:

| Lymphocytes       | Number | Mean (%) | SD   | CV (%) |
|-------------------|--------|----------|------|--------|
| CD69 <sup>+</sup> | 12     | 98.94    | 0.15 | 0.15   |

Cytometer n° 2:

| Lymphocytes       | Number | Mean (%) | SD   | CV (%) |
|-------------------|--------|----------|------|--------|
| CD69 <sup>+</sup> | 12     | 99.66    | 0.25 | 0.26   |

### LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence spillover have not been correctly compensated for and if the regions have not been carefully positioned.
2. It is preferable to use a RBC lysis technique with a washing step as this reagent has not been optimized for "no wash" lysis techniques.
3. Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the technical insert leaflet and compatible with good laboratory practices.
4. The conjugated antibody of this reagent is calibrated so as to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/sample volume ratio in every test.
5. In the case of a hyperleucocytosis, dilute the specimen in PBS so as to obtain a value of approximately 5 x 10<sup>9</sup> leucocytes/L.
6. In certain disease states, such as severe renal failure or haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is recommended to isolate mononucleated cells using a density gradient (Ficoll, for example) prior to staining.

### MISCELLANEOUS

See the Appendix for examples and references.

### TRADEMARKS

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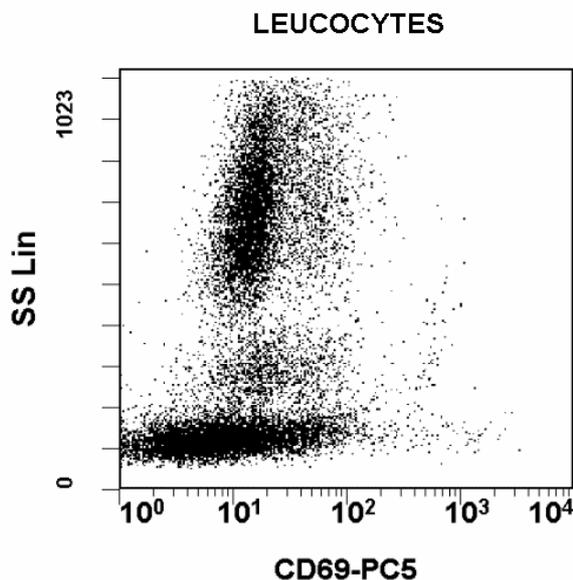


## APPENDIX TO REF IM2656

### EXAMPLES

The graph below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of lyzed normal whole blood sample. Staining is with IOTest CD69-PC5 Conjugated Antibody (Ref. IM2656). All leucocytes are represented.

Analysis is performed with a CYTOMICS FC 500 flow cytometer equipped with CXP Analysis Software.



### REFERENCES

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