

**IOtest®**  
**Lambda-FITC /**  
**CD19-PE**

**REF** A07741  
 50 tests; 1 mL  
 20 µL / test



IOtest  
 Conjugated Antibodies



| ENGLISH               | Specification of constituent 1                          | Specification of constituent 2    |
|-----------------------|---|-----------------------------------|
| <b>Specificity</b>    | Lambda chain  | CD19                              |
| <b>Clone</b>          | Polyclonal  | J4.119                            |
| <b>Hybridoma</b>      | Not applicable  | NS1 x Balb/c                      |
| <b>Immunogen</b>      | Lambda light polyclonal chains                          | Lymphoma cells SKLY18             |
| <b>Immunoglobulin</b> | F(ab') <sub>2</sub>                                     | IgG1                              |
| <b>Species</b>        | Rabbit  | Mouse                             |
| <b>Source</b>         | Serum   | Ascites                           |
| <b>Purification</b>   | Chromatography  | Protein A affinity chromatography |
| <b>Fluorochrome</b>   | Fluorescein isothiocyanate (FITC)                       | R Phycocerythrin (PE)             |
| <b>λ excitation</b>   | 488 nm  | 488 nm                            |
| <b>Emission peak</b>  | 525 nm  | 575 nm                            |
| <b>Buffer</b>         | PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN <sub>3</sub> |                                   |

**USE**

This fluorochrome-conjugated antibody mixture permits the identification and numeration of cell populations expressing CD19 antigen and Lambda light surface immunoglobulin chains present in human biological samples using flow cytometry

**PRINCIPLE**

This test is based on the ability of specific antibodies to bind to the antigenic determinants expressed by leucocytes on the surface. 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 analyzes light diffusion and the fluorescence of cells. The flow cytometer analyzes light diffusion and the fluorescence of cells. It makes possible the localization of cells within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the fluorescence of PE, corresponding to CD19 staining. Other histograms combining two of the different parameters available on the cytometer are also used in the gating stage. The fluorescence of the 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 distinction between secondary hyperlymphocytosis and lymphoproliferative syndromes can be made from the ratio of cells expressing Kappa / cells expressing Lambda. In B-secondary populations, the Kappa / Lambda ratio is between 1/1 and 2/1, whilst in clonal B proliferations, it is usually greater than 3/1, or less than 1/2 (1). The Kappa / Lambda ratio can be obtained by using the two Kappa-FITC/CD19-PE (Ref. A07740) and Lambda-FITC/CD19-PE (Ref. A07741) reagents for the same sample.

The analysis of B monoclonality is useful in the diagnosis of the following diseases: chronic lymphoid leukaemias (CLL), small cell lymphomas, prolymphocyte leukaemias, mantle-cell lymphomas, follicular lymphomas and marginal and associated lymphomas as well as hairy-cell leukaemias (2 – 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 opened 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 take internally and avoid all 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 20, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- IOtest Kappa-FITC/CD19-PE reagent (Ref. A07740).
- Red cell lysis reagent with washing stage after lysis. For example: IOtest 3 Lysis Solution (Ref. A07799).

- Leucocyte fixation reagent. For example: IOtest 3 Fixative Solution (Ref. A07800).
- Isotypic control: IOtest reagent. IgG1-FITC / IgG1-PE (Ref. A07794).
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

**PROCEDURE**

**NOTE:** For each sample analyzed, it is necessary to reduce the non-specific fixation generated by the excess of circulating immunoglobulins by carrying out a prior washing stage (Steps 1 to 3). 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 an isotypic control IOtest (Ref. A07794).

1. Put 100 µL of total blood sampled in EDTA (or 5 x 10<sup>5</sup> cells) into each tube.
2. Add 3 mL of PBS to each tube and vortex vigorously for 3 to 5 seconds.
3. Centrifuge for 5 minutes at 300 x g at room temperature (18 – 25°C), then remove the supernatant by aspiration.
4. Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used. By example, if one wishes to use the IOtest 3 Lysis Solution (Ref. A07799): Add 2 mL of this reagent at its working concentration (1X). Vortex immediately 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 300 x g at room temperature. Then remove the supernatant by aspiration and resuspend the cell pellet using 3 mL of PBS:
6. Again centrifuge for 5 minutes at 300 x g at room temperature. Then remove the supernatant by aspiration and resuspend the cell pellet in 100 µL of PBS:
7. Add 20 µL of the specific IOtest conjugated antibody solution to the test tube.
8. Add 20 µL of the negative control to the control tube. Vortex the tubes gently.
9. Incubate for 15 to 20 minutes at room temperature protected from light.
10. Add 3 mL of PBS and centrifuge for 5 minutes at 300 x g at room temperature.
11. Remove the supernatant by aspiration.

Note: If the preparations are to be kept for more than 2 hours prior to cytometric analysis, or if they require fixation, it is advisable to carry out the following steps 12 to 15. If not, carry out steps 16 and 17 below.

12. Resuspend the cell pellet using 3 mL of PBS.
13. Centrifuge for 5 minutes at 300 x g at room temperature.
14. Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 mL or 1 mL of PBS made up with 0.8% of formaldehyde or for example in the IOTest 3 Fixative Solution (Ref. A07800) at its working concentration (1X).
15. In all cases, keep the preparations between 2 and 8°C and protected from light.

**IMPORTANT:** The tubes thus prepared are ready for cytometric analysis, but cannot be stored for more than 24 hours between 2 and 8°C.

16. Resuspend the cell pellet in 0.5 mL or 1 mL of PBS without formaldehyde.
17. In all cases, keep the preparations between 2 and 8°C and protected from light.

**IMPORTANT:** The tubes thus prepared are ready for cytometric analysis, but cannot be stored for more than two hours between 2 and 8°C.

## PERFORMANCE SPECIFICITY

Anti-Lambda polyclonal antibodies recognize the Lambda light chain of immunoglobulins expressed at the surface of a cellular sub-population corresponding to approximately 1/3 mature B lymphocytes in peripheral blood. This light chain is also found on the surface of a sub-population of bone marrow immature B lymphocytes (8).

The CD19 molecule is expressed on the surface of all B-cell lines, from early pre-B-cells to mature B lymphocytes. Its expression is lost on differentiation into plasmocytes (8-10). The CD19 molecule is not expressed by T lymphocytes or by NK cells, or by monocytes or granulocytes (3).

MaB J4.119 was assigned to CD19 during the 4<sup>th</sup> HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Vienna, Austria, in 1989 (WS Code: B191, Section B) (11).

## LINEARITY

To test the linearity of staining for the specificities of this reagent, a positive cell line (NAMALWA) and a negative cell line (HPBALL) were mixed in different proportions with a constant final number of cells, so that the positive line/negative 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> ) |
|-------------|-------------------|-----------------------------|
| Lambda      | Y = 0.97 X + 1.41 | 0.9987                      |
| CD19        | Y = 0.99 X + 0.42 | 0.9992                      |

## 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 25 healthy adults were treated using the reagent described above. The results obtained for the count (%) of the positive events of interest with this reagent are given in the tables below:

| Lymphocytes             | Number | Mean (%) | SD   | CV (%) |
|-------------------------|--------|----------|------|--------|
| CD19 <sup>+</sup>       | 25     | 5.26     | 3.64 | 69.18  |
| Lambda <sup>+</sup> (*) | 25     | 44.95    | 7.28 | 16.20  |

(\*) Lambda<sup>+</sup> gated on CD19<sup>+</sup> cells

## INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of staining of a positive target were carried out on a target population (leucocyte sub-populations). The results obtained are summarized in the following table:

| Positive Target Lymphocytes | Number | Mean (%) | SD   | CV (%) |
|-----------------------------|--------|----------|------|--------|
| CD19 <sup>+</sup>           | 12     | 2.94     | 0.42 | 14.4   |
| Lambda <sup>+</sup> (*)     | 12     | 44.15    | 3.35 | 7.6    |

(\*) Lambda<sup>+</sup> gated on CD19<sup>+</sup> cells

## INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same positive target (leucocyte sub-populations), 12 measurements of the percentage of stained cells were carried out by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n° 1:

| Positive Target Lymphocytes | Number | Mean (%) | SD   | CV (%) |
|-----------------------------|--------|----------|------|--------|
| CD19 <sup>+</sup>           | 12     | 2.94     | 0.42 | 14.4   |
| Lambda <sup>+</sup> (*)     | 12     | 44.15    | 3.35 | 7.6    |

(\*) Lambda<sup>+</sup> gated on CD19<sup>+</sup> cells

Cytometer n° 2:

| Positive Target Lymphocytes | Number | Mean (%) | SD   | CV (%) |
|-----------------------------|--------|----------|------|--------|
| CD19 <sup>+</sup>           | 12     | 3.27     | 0.34 | 10.5   |
| Lambda <sup>+</sup> (*)     | 12     | 45.11    | 1.88 | 4.2    |

(\*) Lambda<sup>+</sup> gated on CD19<sup>+</sup> cells

## LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence leaks 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 antibodies of this reagent are 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 blood 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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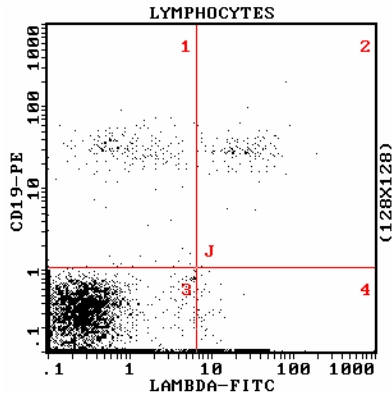
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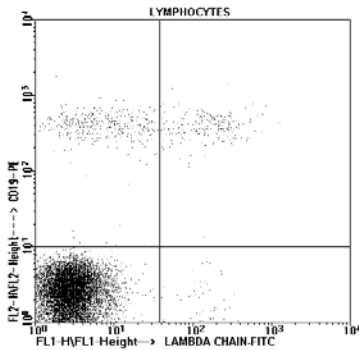
**EXAMPLES**

The graphs below are biparametric representations (Fluorescence Intensity vs. Fluorescence Intensity) of lysed whole blood sample from an healthy donor. Staining is with IOTest Lambda-FITC/CD19-PE Conjugated Antibody (Ref. A07741). Gate is on lymphocytes. The IgG1-FITC/IgG1-PE isotypic control staining (Ref. A07794) is not shown.

Acquisition and analysis are performed with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software:



Acquisition is performed with a Becton Dickinson FACScan™ flow cytometer. Analysis is with LYSYS II™ software:



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