**IOTest®3**
**CD103-FITC /**
**CD11c-PE /**
**CD19-ECD**

**REF A07712**
25 tests; 0.5 mL
20 µL / test

**IOTest 3**
**Conjugated Antibodies**

IVD

| ENGLISH | Specifications of constituent 1 | Specifications of constituent 2 | Specifications of constituent 3 |
|---|---|---|---|
| **Specificity** | CD103 | CD11c | CD19 |
| **Clone** | 2G5 | BU15 | J3-119 |
| **Hybridoma** | P3-X63-Ag.8.653 x Balb/c | NS1/Ag4.1 x Balb/c | NS1 x Balb/c |
| **Immunogen** | Human intestine intra-epithelial lymphocytes | Synovial fluid dendritic cells | Lymphoma cells SKLY 18 |
| **Immunoglobulin** | IgG2a | IgG1 | IgG1 |
| **Species** | Mouse | Mouse | Mouse |
| **Source** | Ascites | Ascites | Ascites |
| **Purification** | Protein A affinity chromatography | Chromatography | Protein A affinity chromatography |
| **Fluorochrome** | Fluorescein isothiocyanate (FITC) | R Phycoerythrin (PE) | R Phycoerythrin-Texas Red®-X (ECD™) |
| **λ excitation** | 488 nm | 488 nm | 488 nm |
| **Emission peak** | 525 nm | 575 nm | 613 nm |
| **Buffer** | PBS pH 7.2 plus 2 mg / mL BSA and 0.1% $NaN_3$ | | |

## USE

This fluorochrome-conjugated antibody mixture is suitable for multiparametric analysis using flow cytometry. It permits the detection of the expression of CD103, CD11c and CD19 antigens in leucocytes.

## 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 3 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. 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) with the fluorescence of ECD, corresponding to CD19 staining. Other histograms combining two of the different parameters available on the cytometer are also used in the gating stage. The cell population thus gated is subdivided into sub-populations, using the two other fluorescences.
In this way, the positively-stained cells are distinguished from the unstained cells. The results are expressed as a percentage of fluorescent cells in relation to all the events acquired by the gating.

## EXAMPLES OF CLINICAL APPLICATIONS

Analysis of the expression of antigens CD103, CD11c and CD19 is useful for the identification and the characterization of B leukaemias, which express these antigens (1 – 6). In particular, the expression of CD103 is considered as the most reliable marker in distinguishing hairy-cell leukaemias from other B leukaemias (7). Although expression of CD11c is not specific for hairy-cell leukaemias, expression of CD25 and CD11c associated with an absence of expression of CD23 and CD5, may confirm the diagnosis (1, 6). This complementary analysis can be performed with the help of the following IOTest 3 reagents: CD5-FITC / CD23-PE / CD19-ECD (Ref. A07710) and CD25-FITC / CD11c-PE / CD19-ECD (Ref. A07711). Finally, analysis of the expression of antigens CD103, CD11c and CD19 is useful for the diagnosis of certain non-Hodgkin lymphomas (1, 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 ($NaN_3$) 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.

## SPECIMENS

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 samples 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).
- To obtain optimal results, the following reagents are recommended:
  - Lysing reagent: IOTest 3 Lysis Solution (Ref. A07799).
  - Fixation reagent: IOTest 3 Fixative Solution (Ref. A07800).
  - One of the following IOTest 3 negative controls:
    Neg.Ctrl.-FITC/Neg.Ctrl.-PE/CD19-ECD (Ref. A07730) or
    Neg.Ctrl.-FITC/Neg.Ctrl.-PE/Neg.Ctrl.-ECD (Ref. A07732).
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

### PROCEDURE

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed with the IOTest 3 negative control (Ref. A07730 or A07732).

1. Add 20 µL of specific IOTest 3 conjugated antibodies to each test tube, and 20 µL of the appropriate negative control to each control tube.
2. Add 100 µL of the test sample (i.e., the equivalent of approximately 5 x $10^5$ cells). Vortex the tubes gently.
3. Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4. Then perform, if necessary, lysis of the red cells by adding 2 mL of IOTest 3 Lysis Solution (Ref. A07799) 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.
6. Remove the supernatant by aspiration.
7. Resuspend the cell pellet using 3 mL of PBS.
8. Repeat stage 5.
9. Remove the supernatant by aspiration and resuspend the cell pellet using:
   - 0.5 mL or 1 mL of IOTest 3 Fixative solution (Ref. A07800) at its working concentration (1X), if the preparations are to be kept for more than 2 hours and for less than 24 hours,
   - 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 CD103 antigen (integrin αE sub-unit / surface antigen HML-1) is a type I transmembrane protein, expressed in over 90% of intra-epithelial lymphocytes and in approximately 40% of T lymphocytes from the intestinal "lamina propria" (8, 9). Its expression is found in the majority of intra-epithelial lymphocytes present in other tissues such as bronchi and skin and - in inflammatory conditions - mammary tissue, the salivary glands and tumoral epithelia (8, 9). In peripheral blood and in peripheral lymphoid organs, the CD103 antigen is expressed on 0.5 to 5% of normal lymphocytes.

The 2G5 monoclonal antibody (mAb) was assigned to CD103 during the 5th HLDA Workshop on Human Leucocyte Differentiation Antigens, Boston, USA, in 1993 (WS Code: A005, Section A; WS Code:29, Section BP) (10).

The CD11c antigen (integrin αX subunit / surface antigen p150) is a type I transmembrane protein, which is expressed mainly by monocytes, macrophages and NK (Natural Killer) cells, and to a lesser extent by granulocytes, dendritic cells and certain T and B lymphocyte sub-populations (11).

MAb BU15 was assigned to CD11c during the 3rd HLDA Workshop in Oxford, England, in 1986 (WS Code: 256, Section M) (12).

The CD19 molecule is expressed on the surface of all B-lymphocyte cell lines, from early pre-B-cells to mature B lymphocytes. Its expression is lost on differentiation into plasmocytes (13-15). The CD19 molecule is not expressed by T lymphocytes or by NK cells, or by monocytes or granulocytes (15).

MAb J3-119 was assigned to CD19 during the 4th HLDA Workshop in Vienna, Austria, in 1989 (16, 17).

### LINEARITY

In order to test the linearity of this reagent, suitable targets were chosen for each stain. For CD103 and CD19, one pair of cell lines (one positive, one negative): MOLT16 [CD103$^+$ CD11c$^-$CD19$^-$] and DAUDI [CD103$^-$CD11c$^-$ CD19$^+$]. For CD11c, the above negative cell lines with a positive leucocyte target: granulocytes [CD103$^-$CD11c$^+$CD19$^-$]. These cell populations were mixed in different proportions with a constant final number of cells, such that the positive to negative cell ratio of the mixtures 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 ($R^2$) |
|---|---|---|
| CD103 | Y = 0.97 X + 1.36 | 0.998 |
| CD11c | Y = 1,01 X + 0,44 | 0.998 |
| CD19 | Y = 0.95 X + 1.71 | 0.997 |

### 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, samples of the whole blood of 50 healthy adults were treated using the reagent described above. Figures in relation to CD103 are not given, as only a small population of normal lymphocytes (0.5 to 5%) express the CD103 antigen. The mean values of the results obtained in the leucocyte sub-populations of interest in these 50 donors are shown in the tables below:

| Lymphocytes | Number | Mean (%) | SD | CV (%) |
|---|---|---|---|---|
| CD11c$^+$ | 50 | 19.57 | 6.73 | 34.39 |
| CD19$^+$ | 50 | 9.87 | 4.96 | 50.24 |

| Monocytes | Number | Mean (%) | SD | CV (%) |
|---|---|---|---|---|
| CD11c$^+$ | 50 | 96.44 | 2.56 | 2.65 |

| Granulocytes | Number | Mean (%) | SD | CV (%) |
|---|---|---|---|---|
| CD11c$^+$ | 50 | 99.13 | 0.66 | 0.67 |

### INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of positive target cells (mixture of Control Cells CYTO-TROL™ (Ref. 6604248) + MOLT16 human cell line) were carried out. The results obtained are summarized in the following table:

| CYTO-TROL mixture + MOLT16 | Number | Mean (%) | SD | CV (%) |
|---|---|---|---|---|
| CD103$^+$ | 12 | 24.34 | 0.59 | 2.4 |
| CD11c$^+$ | 12 | 14.98 | 0.30 | 2.0 |
| CD19$^+$ | 12 | 9.55 | 0.20 | 2.1 |

### INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same mixture of positive targets (mixture of CYTO-TROL + MOLT16 human cell line), 12 measurements of the percentage of positive factors were undertaken by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n°1:

| CYTO-TROL mixture + MOLT16 | Number | Mean (%) | SD | CV (%) |
|---|---|---|---|---|
| CD103$^+$ | 12 | 24.34 | 0.59 | 2.4 |
| CD11c$^+$ | 12 | 14.98 | 0.30 | 2.0 |
| CD19$^+$ | 12 | 9.55 | 0.20 | 2.1 |

Cytometer n°2:

| CYTO-TROL mixture + MOLT16 | Number | Mean (%) | SD | CV (%) |
|---|---|---|---|---|
| CD103$^+$ | 12 | 24.87 | 0.54 | 2.2 |
| CD11c$^+$ | 12 | 13.50 | 0.35 | 2.6 |
| CD19$^+$ | 12 | 8.63 | 0.28 | 3.3 |

## 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 conjugated 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 $10^9$ 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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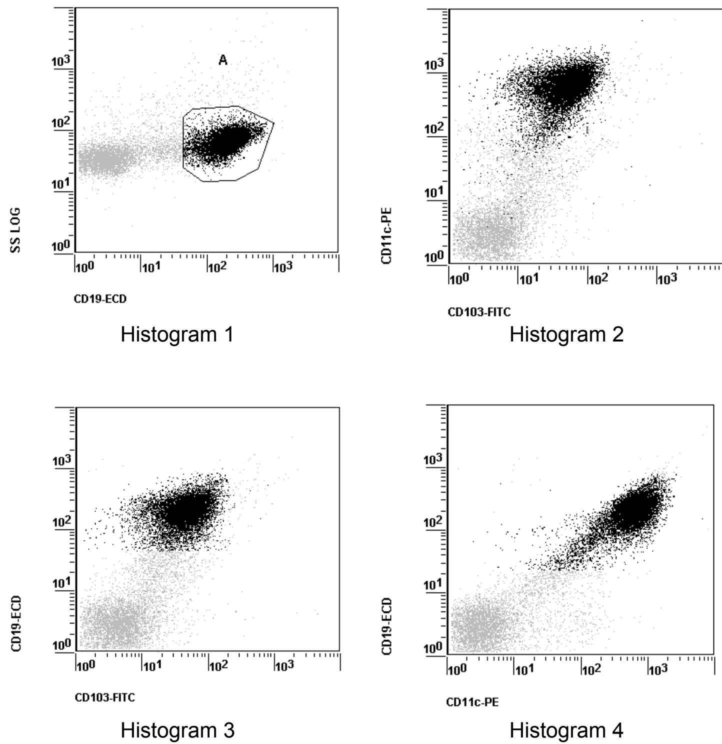
# APPENDIX TO REF A07712

## EXAMPLES

The 4 diagrams below are biparametric representations (Side Scatter *versus* Fluorescence Intensity or Fluorescence Intensity *versus* Fluorescence Intensity) of a Hairy Cell Leukemia specimen (HCL). A peripheral blood sample is stained with CD103-FITC / CD11c-PE / CD19-ECD Conjugated Antibodies (Ref. A07712). Lysis and fixation are with IOTest 3 Lysing Solution (Ref. A07799) and IOTest 3 Fixative Solution (Ref. A07800) respectively. All acquired events are shown. Region A defines the gating strategy (CD19 positive cluster) used on this example. Gated events are shown in dark in all histograms.

Acquisition is with a COULTER® EPICS® XL™ Flow Cytometer equipped with System II™ Software. Analysis is with EXPO™ Cytometer Software (Ref. 6605434).




Histogram 1 Histogram 2

Histogram 3 Histogram 4

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