**APPLICATIONS**

This fluorochrome-conjugated antibody permits the identification and enumeration of cell populations expressing the CD10 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 leukocytes. 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 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**

Analysis of the expression of the CD10 antigen is found to be useful, in combination with other markers, in the characterization of different leukemias (1). The simultaneous analysis of CD20, CD10 and CD19 antigens helps the characterization of different B-cell neoplasias (2), such as chronic B-cell lymphoid leukemias (CLL-B) and small-cell lymphomas, which are CD20+CD10+, and follicular lymphomas, which are CD20+CD10+ (3, 4). This combination may also help to differentiate between acute B-cell lymphoblastic leukemias (ALL-B) and pre-B-cell ALLs, which are CD20+CD10+, polytypic or polyphenotypic leukemias, which are CD20+CD10− (2). For ALL-Bs, two subgroups can be distinguished, the ALL-B CD10+ and the ALL-B CD10−, the first group having a more favourable prognosis (3).

**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.
5. Avoid microbial contamination of the reagents, or false results may occur.

**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.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- Red cell lysis reagent with washing stage after lysis. For example:

**NOTE**

In all cases, keep the preparations between 2 and 8°C and protected from light.
PERFORMANCE SPECIFICITY
The monoclonal antibody ALB1 was studied during the 1st HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Paris, France, 1982 (5).

LINEARITY
To test the linearity of staining of this reagent, a positive cell line (RAMOS) and a negative cell line (MOLT4) 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.

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Linear regression</th>
<th>Linearly (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>Y = 0.99 X + 0.69</td>
<td>0.9996</td>
</tr>
</tbody>
</table>

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 50 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:

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>50</td>
<td>1.50</td>
<td>2.14</td>
<td>143</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>Number</td>
<td>Mean (%)</td>
<td>SD</td>
<td>CV</td>
</tr>
<tr>
<td>CD10</td>
<td>50</td>
<td>95.31</td>
<td>3.56</td>
<td>3.73</td>
</tr>
</tbody>
</table>

INTRA-LABORATORY REPRODUCIBILITY
On the same day and using the same cytometer, 12 measurements of the percentage of staining of a positive target (lymphocytes) were carried out. The results obtained are summarized in the following table:

<table>
<thead>
<tr>
<th>Positive Target</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes CD10⁺</td>
<td>12</td>
<td>1.22</td>
<td>0.18</td>
<td>14.6</td>
</tr>
</tbody>
</table>

INTER-LABORATORY REPRODUCIBILITY
On the same day and for the same positive target (lymphocytes), 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:

<table>
<thead>
<tr>
<th>Positive Target</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes CD10⁺</td>
<td>12</td>
<td>1.22</td>
<td>0.18</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Cytometer n° 2:

<table>
<thead>
<tr>
<th>Positive Target</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes CD10⁺</td>
<td>12</td>
<td>1.32</td>
<td>0.17</td>
<td>13.0</td>
</tr>
</tbody>
</table>

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 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 blood in PBS so as to obtain a value of approximately 5 x 10⁹ 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 A07761

EXAMPLES

The graphs below are biparametric representations (SS vs. Fluorescence Intensity) of lyzed normal whole blood sample. Staining is with IOTest CD10-PC5 Conjugated Antibody (Ref. A07761). Gate is on leucocytes.

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

REFERENCES