<p>Stability of closed vial: see expiry date on vial.

**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 aids in the electronic 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 electronic gating.

**EXAMPLES OF CLINICAL APPLICATIONS**

The CD8 antigen is expressed on approximately 30% of total peripheral blood lymphocytes in healthy individuals (1), on 80% of thymocytes (2) and on a sub-population representing 15 to 30% of bone marrow lymphocytes (3). In peripheral blood, this antibody identifies cytotoxic T cell lymphocytes / Suppressors (Tc) as well as a sub-population of NK cells. The latter express the CD8 antigen at a lesser concentration in 1 mL of PBS). 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 purging it into the drainage system so as to avoid the accumulation of sodium azide in metal pipes and to prevent the risk of explosion.

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).

Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.

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 samples should be homogenized by gentle agitation prior to taking the test sample. The samples should be analyzed within 24 hours of venipuncture.

**METHODOLOGY**

**NECESSARY MATERIAL NOT SUPPLIED**

- Flow cytometer.
- Automatic agitator (Vortex type).
- Buffer (PBS: pH 7.2 plus 2 mg / mL BSA and 0.1% NaN₃).
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic pipettes with disposable tips for sampling.
- Plastic haemolysis tubes.
- Calibration beads. For example: APC (675/633) Set-up Kit (Ref. 6607120).
- 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: IOTest® reagent. IgG1-APC (Ref. IM2475).
- Fixative Solution (Ref. A07800).
- Venous blood or bone marrow samples.
- Sampling tubes and material necessary for sampling.
- Venous blood or bone marrow samples.
- Centrifuge for 5 minutes at 150 x g at room temperature.
- Resuspend the cell pellet using 3 mL of PBS.
- Automatic agitator (Vortex type).
- Bedoukian & Co.
- 1. Add 0.5 mL of IOTest® conjugated antibody to each test tube, and 10 µL 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. Replace step 5.
- 8. 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.

<table>
<thead>
<tr>
<th>ENGLISH</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>CD8</td>
</tr>
<tr>
<td>Clone</td>
<td>B9.11</td>
</tr>
<tr>
<td>Hybridoma</td>
<td>NS1 x Balb/c</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Human cytotoxic T-lymphocyte clone (HLA A2)</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>IgG1</td>
</tr>
<tr>
<td>Species</td>
<td>Mouse</td>
</tr>
<tr>
<td>Source</td>
<td>Ascites</td>
</tr>
<tr>
<td>Purification</td>
<td>Protein A affinity chromatography</td>
</tr>
<tr>
<td>Fluorochrome</td>
<td>Phycoerythrin (PE)</td>
</tr>
<tr>
<td>λ excitation</td>
<td>633 nm</td>
</tr>
<tr>
<td>Emission peak</td>
<td>675 nm</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN₃</td>
</tr>
</tbody>
</table>
PERFORMANCE

SPECIFICITY
The B9.11 monoclonal antibody (mAb) reacts with the α sub-unit of the CD8 heterodimer (2). MAb B9.11 was assigned to CD8 during the 1st HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Paris, France, in 1982 (WS Code: 43, Section T) (10).

LINEARITY
To test the linearity of staining for this reagent, a positive cell line (HPBALL) and a negative cell line (DAUDI) 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.

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Linear regression</th>
<th>Linearity (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8</td>
<td>Y = 0.9946 X - 0.8505</td>
<td>0.9992</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 used. The results obtained for the count of the positive events of interest 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>CD8</td>
<td>50</td>
<td>16.11</td>
<td>5.6</td>
<td>34.7</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8</td>
<td>12</td>
<td>36.13</td>
<td>0.54</td>
<td>1.50</td>
</tr>
</tbody>
</table>

INTER-LABORATORY REPRODUCIBILITY
On the same day and on the same sample containing positive cells (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 tables:

Cytometer n° 1:

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8</td>
<td>12</td>
<td>36.13</td>
<td>0.54</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Cytometer n° 2:

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8</td>
<td>12</td>
<td>36.96</td>
<td>0.58</td>
<td>1.57</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 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 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 IM2469

EXAMPLES
The graph below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of lysed normal whole blood sample. Staining is with IOTest CD8-APC Conjugated antibody (Ref IM2469).

All leucocytes are represented.
Analysis is performed with CYTOMICS FC 500 equipped with CXP Software.

REFERENCES