**USE**

This fluorochrome-conjugated antibody permits the identification and enumeration of cell populations expressing the CD3 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 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 unstrained 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 CD3 antigen is a protein complex, which consists of 5 polypeptide chains (γ, δ, ε, ζ, η) linked to TCR (1, 2). The CD3 antigen is expressed only on mature T lymphocytes and on a sub-population of thymocytes (3). In peripheral blood, approximately 67 to 76% of lymphocytes are CD3⁺; this percentage is lower in young children and varies according to age (4).

This reagent enables the characterization and enumeration of T lymphocytes in immune system disorders: immune deficiencies, auto-immune disorders, hypersensitivity reactions, viral infections, restoration of the immune response after bone marrow and/or organ transplantation. In malignant blood dyscrasias such as leukaemias and lymphomas, it permits the follow-up and phenotyping of CD3⁺ populations (5 - 8).

**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.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. Antibody solutions containing sodium azide (NaN₃) 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 reagents, or false results may occur.

The samples should be kept at room temperature (18 – 25°C) and not shaken. The samples should be kept at room temperature and protected from light.

The samples can be kept for up to 90 days from the date of collection. If the sample does not contain red cells, add 2 mL of PBS.

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.

**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.
- Calibrations beads. For example: APC (675/633) Set-up Kit (Ref. 6607120).
- Red blood cell lysis reagent with washing stage after lysis. For example: VersaLyse (Ref. A09777).
- Leucocyte lysis reagent. For example: IOTest 3 Fixative Solution (Ref. A07800).
- Isotypic control: IOTest reagent.

**NOTE**

In all cases, keep the preparations between 2 and 8°C and protected from light.
PERFORMANCE SPECIFICITY

The monoclonal antibody (mAb) UCHT1 reacts with the ε chain of the CD3 complex (9). UCHT1 mAb was assigned to CD3 during the 1st HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Paris, France, in 1982 (WS Code: 3, Section T) (10).

LINEARITY

To test the linearity of staining of 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>CD3</td>
<td>Y = 0.9887 X + 1.2263</td>
<td>0.9995</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>CD3+</td>
<td>50</td>
<td>72.7</td>
<td>7.18</td>
<td>10</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>CD3+</td>
<td>12</td>
<td>41.27</td>
<td>0.64</td>
<td>1.55</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>CD3+</td>
<td>12</td>
<td>56.7</td>
<td>0.64</td>
<td>1.09</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>CD3+</td>
<td>12</td>
<td>62.1</td>
<td>0.79</td>
<td>1.28</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 IM2467

EXAMPLES
The graph below is a biparametric representation (Side Scatter vs. Fluorescence Intensity) of lysed normal whole blood sample. Staining is with IOTest CD3-APC Conjugated Antibody (Ref. IM2467). All leucocytes are represented.

Analysis is performed with CYTOMICS FC 500 equipped with CXP Software.

REFERENCES