

**IOTest®**  
**TdT (Pool)-**  
**FITC**

**REF** IM3524  
 50 tests; 1 mL  
 20 µL / test



**IOTest**  
**Conjugated Antibody**



ENGLISH	Specifications
<b>Specificity</b>	TdT (Pool)
<b>Clone</b>	HT1, HT4, HT8, HT9
<b>Hybridoma</b>	SP2 x Balb/c
<b>Immunogen</b>	Human TdT, bovine TdT
<b>Immunoglobulin</b>	IgG1, IgG2a
<b>Species</b>	Mouse
<b>Source</b>	Ascites
<b>Purification</b>	Protein A affinity chromatography
<b>Fluorochrome</b>	Fluorescein isothiocyanate (FITC)
<b>λ excitation</b>	488 nm
<b>Emission peak</b>	525 nm
<b>Buffer</b>	PBS pH 7.2 plus 6 mg / mL BSA and 0.1% NaN <sub>3</sub>

**USE**

This fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the TdT (Pool) 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 on the internal surface of the plasma membrane, in the cytoplasm or in the nucleus.

Following a membrane fixation/permeabilization step, internal staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The leucocytes are then 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**

TdT is expressed in the early stages of T and B lymphocyte development in normal thymus and bone marrow. Elevated levels of TdT have also been found in neoplasms derived from immature lymphoid cells. Increased enzyme activity has been reported in the majority of acute lymphoblastic leukemias and is also detected in a variable proportion of myeloid leukemias (1-3).

**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 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.
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 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 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, 50 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads. For example: Flow-Set™ Fluorospheres (Ref. 6607007).
- To obtain optimal results, the following reagents are recommended:
  - IntraPrep™ Fixation/Permeabilization reagent (Ref. A07802 or A07803).
  - Leucocyte fixation reagent. For example: IOTest 3 Fixative Solution (Ref. A07800).
- Isotypic controls: IOTest reagents IgG2a-FITC (Ref. A12689) and IgG1-FITC (Ref. A07795).
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

**PROCEDURE**

For the following procedure, the use of the IntraPrep Fixation/Permeabilization reagent (Ref. A07802 or A07803) is recommended.

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed with the isotypic controls IgG2a-FITC (Ref. A12689) and IgG1-FITC (Ref. A07795).

For a blood sample, optimal staining is obtained using a number of leucocytes between 3 and 10 x 10<sup>3</sup> cells / µL. If the leucocyte concentration is greater than 10 x 10<sup>3</sup> cells / µL, dilute. In the case of a cell suspension, optimal staining is obtained with 5 x 10<sup>3</sup> cells / µL; dilute if necessary.

1. Add 50 µL of blood sampled into EDTA or 5 x 10<sup>5</sup> cells to 2 tubes (one test tube, one control tube).
2. Add to each tube 100 µL of IntraPrep reagent 1 (Fixation).
3. Vortex the tubes vigorously for 3 to 5 seconds.
4. Incubate for 15 minutes at room temperature (18 – 25°C) protected from light.
5. Add 4 mL of PBS to each tube.
6. Centrifuge for 5 minutes at 300 x g at room temperature. Remove the supernatant by aspiration.
7. Add to each tube 100 µL of IntraPrep reagent 2 (Permeabilization). Let mix by diffusion. DO NOT VORTEX.
8. Incubate for 5 minutes at room temperature WITHOUT SHAKING.
9. Shake the tubes carefully and manually for 2 to 3 seconds.
10. Add 20 µL of the specific IOTest conjugated antibody to each test tube, and 20 µL of each isotypic control to the control tube. Vortex the tubes gently.
11. Incubate for 15 to 20 minutes at room temperature protected from light.
12. Add 4 mL of PBS and centrifuge at 300 x g for 5 minutes at room temperature.
13. Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 to 1 mL of IOTest 3 Fixative Solution (Ref. A07800) at its working concentration (1X).
14. The preparations are ready for cytometric analysis.

NOTE: If the preparations are to be stored for more than 2 hours prior to cytometric analysis, it is advisable to store them at 2-8°C and protected from light. The preparations thus stored do not keep, however, for more than 24 hours.

## PERFORMANCE

### SPECIFICITY

Terminal deoxynucleotidyl Transferase (TdT) is a nuclear enzyme with a molecular weight of 58 kDa (4). This molecule catalyses random addition of nucleotides on the 3' OH termini of single-stranded DNA. TdT differs from other DNA polymerases because it does not require a template sequence for polymerization (4, 5). TdT is mainly expressed during the maturation of the immune system in primary and secondary lymphoid organ cells: i.e. precursors of B-lymphocytes in the bone marrow and precursors of T-lymphocytes in the thymus (4, 6, 7).

TdT expressing cells are never observed in normal peripheral blood. The finding of TdT positive cells in tissues other than bone marrow and thymus is a strong evidence for the involvement of lymphoblastic leukemia / lymphoma. The TdT expression is not found in myeloid, erythroid, or megakaryocytic precursors, or in other non lymphoid cell components, including mesenchymal, epithelial, and germ cells (4). This mixture of 4 monoclonal antibodies (HT1, HT4, HT8 and HT9) is directed against the TdT protein.

### LINEARITY

To test the linearity of staining of this reagent, a positive cell line (MOLT4) and negative cells (Cyto-Trol™ Control Cells (Ref. 6604248) TdT<sup>-</sup>) were mixed in different proportions and with a constant final number of cells, so that the positive line / negative cells 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> )
TdT (Pool)	$Y = 1.0114 X - 2.3079$	0.9989

### 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 permeabilized whole blood samples of 10 healthy adults were used. The results obtained for the count of the negative events of interest are given in the table below:

Negative cells	Number	MFI	SD	CV (%)
TdT <sup>-</sup> Lymphocytes	10	0.25	0.02	9.25
TdT <sup>-</sup> Monocytes	10	0.44	0.05	12.2
TdT <sup>-</sup> Granulocytes	10	0.50	0.13	26.6

### INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the positivity of a sample containing positive cells (mixture of a permeabilized positive cell line MOLT4 and negative cells Cyto-Trol Control Cells) were carried out. The results obtained are summarized in the following table:

Positive Cells	Number	Mean (%)	SD	CV (%)
TdT (Pool) <sup>+</sup>	12	75.40	2.48	3.29

### INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same sample containing positive cells (mixture of a permeabilized positive cell line MOLT4 and negative cells Cyto-Trol Control Cells), 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:

Positive Cells	Number	Mean (%)	SD	CV (%)
TdT (Pool) <sup>+</sup>	12	75.40	2.48	3.29

Cytometer n° 2:

Positive Cells	Number	Mean (%)	SD	CV (%)
TdT (Pool) <sup>+</sup>	12	31.43	1.95	6.21

### 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. 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.
3. 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.
4. In the case of a hyperleucocytosis, dilute the specimen in PBS so as to obtain a value of approximately  $5 \times 10^9$  leucocytes/L.
5. 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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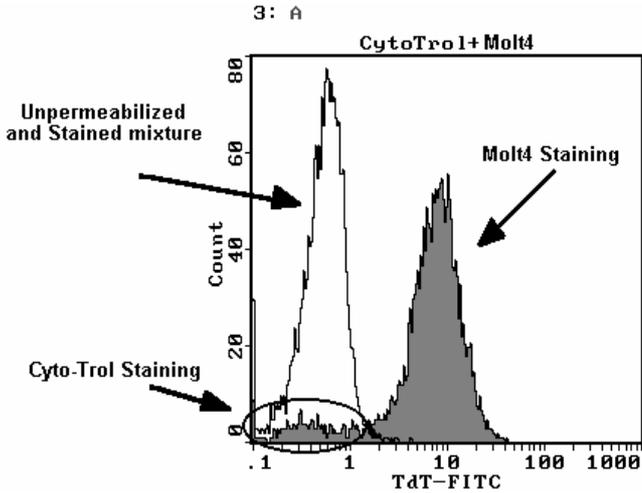


## APPENDIX TO REF IM3524

### EXAMPLE

The diagram shown below is a monoparametric representation (Count versus Fluorescence Intensity) of a permeabilized-mixture of Cyto-Trol Control Cells (PN 6604248) and MOLT4 cell line. Staining is with TdT-FITC mixture of 4 monoclonal antibodies (PN IM3524). Gate is on all the mixture permeabilized with IntraPrep Reagent (Ref. IM2388 and Ref. IM2389).

Cyto-Trol Control Cells do not express TdT, then constituting a negative control (left side of the solid histogram), whereas the Molt4 cell line is known to express TdT (right side of the solid histogram). Unpermeabilized mixture stained with TdT-FITC is underneath in light and shows a similar staining-pattern as the Cyto-Trol.



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

### REFERENCES

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