IOTest®3
Kappa-FITC / Lambda-PE / CD19-ECD

REF A07706
25 tests; 0.5 mL
20 µL / test

BECKMAN COULTER™

IOTest 3
Conjugated Antibodies

IVD CE

ENGLISH	Specifications of constituent 1	Specifications of constituent 2	Specifications of constituent 3
Specificity	Kappa chain	Lambda chain	CD19
Clone	Polyclonal	Polyclonal	J4.119
Hybridoma	N/A	N/A	NS1 x Balb/c
Immunogen	Light polyclonal Kappa chains	Light polyclonal Lambda chains	Lymphoma cells SKLY18
Immunoglobulin	$F(ab')_2$	$F(ab')_2$	IgG1
Species	Rabbit	Rabbit	Mouse
Source	Serum	Serum	Ascites
Purification	Chromatography	Chromatography	Chromatography on Protein A
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	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 the CD19 antigen and light Kappa and Lambda surface immunoglobulin chains in leucocytes.

PRINCIPLE
This test is based on the ability of specific 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 the ECD corresponding to CD19 staining. Other histograms combining two of the different parameters available on the cytometer, are also used in the electronic 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 electronic gating.

EXAMPLES OF CLINICAL APPLICATIONS
The distinction between secondary hyperlymphocytosis and lymphoproliferative syndromes can be made from the ratio of cells expressing Kappa / cells expressing Lambda. In B-secundary populations, the Kappa / Lambda ratio is between 1/1 and 2/1, whilst in clonal B proliferations, it is usually greater than 3/1, or less than 1/2 (1).
The analysis of B monoclonality is useful in the diagnosis of the following diseases : chronic lymphoid leukaemias (CLL), small cell lymphomas, prolymphocyte leukaemias, mantle-cell lymphomas, follicular lymphomas and marginal zone lymphomas as well as hairy-cell leukaemias (2 – 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.
7. 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.
8. 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).
9. Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.
10. 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 taking them.

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, one control tube is required in which the cells are mixed with the IOTest 3 negative control (Ref. A07730 or A07732).
1. Put 100 µL of total blood sampled in EDTA (or 5 x 10^5 cells) into 2 tubes (one test tube, one control tube).
2. Add 3 mL of PBS to each tube and vortex vigorously for 3 to 5 seconds.
3. Centrifuge for 5 minutes at 300 x g at room temperature (18-25°C), then remove the supernatant by aspiration.
4. Then perform 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.
5. Centrifuge for 5 minutes at 300 x g at room temperature. Then remove the supernatant by aspiration and resuspend the cell pellet in 3 mL of PBS.
6. Again centrifuge for 5 minutes at 300 x g at room temperature. Then remove the supernatant by aspiration and resuspend the cell pellet in 100 µL of PBS.
7. Add 20 µL of the specific IOTest 3 conjugated antibody solution to the test tube.
8. Add 20 µL of the negative control to the control tube. Vortex the tubes gently.
9. Incubate for 15 to 20 minutes at room temperature protected from light.
1. Add 3 mL of PBS and centrifuge for 5 minutes at 300 x g at room temperature.
2. Remove the supernatant by aspiration.

   Note: If the preparations are to be conserved for more than 2 hours prior to cytometric analysis, or if they require fixation, it is advisable to carry out the following stages 12 to 15. If not, carry out stages 16 and 17 below.

12. Resuspend the cell pellet using 3 mL of PBS.
13. Centrifuge for 5 minutes at 300 x g at room temperature.
14. 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).

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15. Note: In all cases, keep the preparations between 2 and 8°C and protected from light.

IMPORTANT : The tubes thus prepared are ready for cytometric analysis, but cannot be stored for more than 24 hours between 2 and 8°C.

16. Resuspend the cell pellet in 0.5 mL or 1 mL of PBS without formaldehyde.
17. In all cases, keep the preparations between 2 and 8°C and protected from light.

IMPORTANT : The tubes thus prepared are ready for cytometric analysis, but cannot be stored for more than two hours between 2 and 8°C.

PERFORMANCE

SPECIFICITY

Anti-Kappa polyclonal antibodies recognize the Kappa light chain of immunoglobulins expressed at the surface of a cellular sub-population corresponding to approximately 2/3 of mature B lymphocytes in peripheral blood. This light chain is also found on the surface of a sub-population of immature bone marrow B lymphocytes (8).
Anti-Lambda polyclonal antibodies recognize the Lambda light chain of immunoglobulins expressed at the surface of a cellular sub-population corresponding to approximately 1/3 of mature B lymphocytes in peripheral blood. This light chain is also found on the surface of a sub-population of immature bone marrow B lymphocytes (8).
The CD19 molecule is expressed on the surface of all B-cell lines, from early pre-B-cells to mature B lymphocytes. Its expression is lost upon differentiation into plasmocytes (8-10). The CD19 molecule is neither expressed by T lymphocytes nor by NK cells, nor by monocytes or granulocytes (3). The J4.119 monoclonal antibody was assigned to CD19 during the 4th HLDA Workshop on Human Leucocyte Differentiation Antigens, Vienna, Austria, 1989 (WS Code: B191, Section B) (11).

LINEARITY

To test the linearity of staining for the specificities of this reagent, a positive cell line and a negative cell line (DAUDI for Kappa, NAMLWA for Lambda and CD19) 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. The parameters of the equation of the linear regression may be used to determine the coefficient of linearity as well as the range of measurement for each specificity.

Specificity	Linear regression	Linearity (R^2)	Range (%)
Kappa	Y = 0.94 X + 1.39	0.998	2 – 90
Lambda	Y = 0.94 X + 0.70	0.999	2 – 90
CD19	Y = 0.95 X + 0.11	0.999	1 – 90

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 of 50 healthy adults was treated using the reagent described above. The mean values of the results obtained in the lymphocyte population of these 50 donors are shown in the tables below :

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD19+	50	8.63	3.21	37.2

Lymphocytes CD19+	Number	Mean (%)	SD	CV (%)
Kappa+	50	61.77	6.34	10.3
Lambda+	50	37.58	6.23	16.6

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of positive cells were carried out on a target population (mixture of NAMALWA and DAUDI cell lines). The results obtained are summarized in the following table:

Mixture NAMALWA + DAUDI	Number	Mean (%)	SD	CV (%)
Kappa+	12	54.37	0.38	0.7
Lambda+	12	45.81	0.3	0.7
CD19+	12	99.81	0.03	0.0

INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same population (mixture of NAMALWA and DAUDI cell lines), 12 measurements of the percentage of positive 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 :

Mixture NAMALWA + DAUDI	Number	Mean (%)	SD	CV (%)
Kappa+	12	54.37	0.38	0.7
Lambda+	12	45.81	0.3	0.7
CD19+	12	99.81	0.03	0.0

Cytometer n°2 *:*

Mixture NAMALWA + DAUDI	Number	Mean (%)	SD	CV (%)
Kappa+	12	55.08	0.29	0.5
Lambda+	12	45.78	0.46	1.0
CD19+	12	99.65	0.07	0.1

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. A lysis technique with washing is preferable as this reagent has not been optimized for "without washing" 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 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/number of cells 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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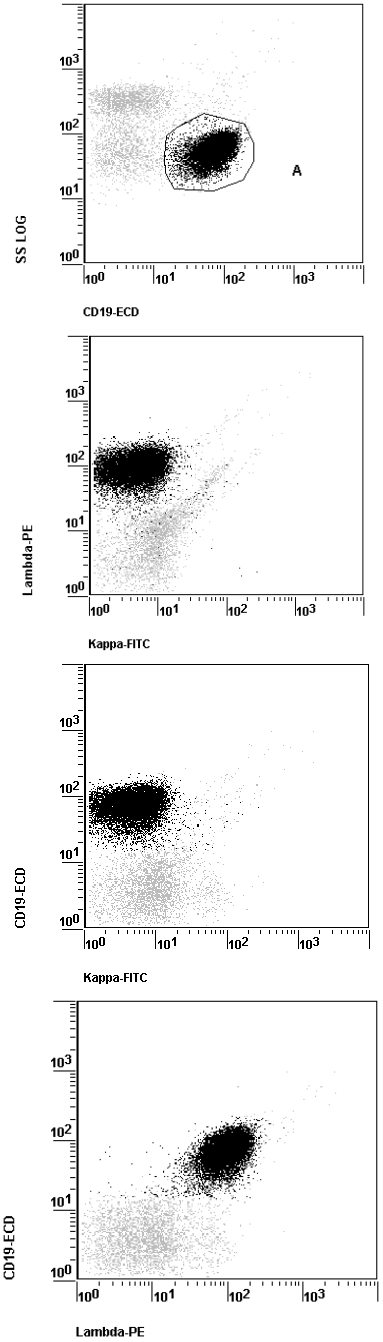
APPENDIX TO REF A07706

EXAMPLES

The 4 diagrams below are biparametric representations (Side Scatter versus Fluorescence Intensity or Fluorescence Intensity versus Fluorescence Intensity) of a Lambda-positive B-CLL specimen (peripheral blood). Staining is with Kappa-FITC / Lambda-PE / CD19-ECD Conjugated Antibodies (Ref. A07706). 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 (CD19 positive) defines the gating strategy 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).



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