IOTest®3
Neg. Ctrl.-FITC /
Neg. Ctrl.-PE /
CD45-ECD

REF A07729
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	Not applicable	Not applicable	CD45
Clone	679.1Mc7	679.1Mc7	J33
Hybrid	P3-X63-Ag.8.653 x Balb/c	P3-X63-Ag.8.653 x Balb/c	NS1 x Balb/c
Immunogen	Non-biological Hapten	Non-biological Hapten	Laz 221 cell line
Immunoglobulin	IgG1	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites	Ascites	Ascites
Purification	Chromatography	Chromatography	Protein A affinity chromatography
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	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN_3		

USE

This fluorochrome-conjugated antibody mixture (Negative Control-FITC / Negative Control-PE / CD45-ECD) from the IOTest 3 range is suitable for flow cytometry multiparametric analysis. It permits the determination of non-specific staining of mixtures of conjugated antibodies belonging to this range on human leucocytes.

PRINCIPLE

These conjugated antibodies have been calibrated for use in direct flow cytometry immuno-staining techniques.
The flow cytometer analyses the fluorescence of cells. It makes possible the localisation of cells within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light ("Side Scatter" or SS) on analysed cells and with the fluorescence of the ECD corresponding to CD45 staining. The results are expressed as a percentage of positive cells for the two other fluorescences (FITC and PE) in relation to all the events acquired by the gating.

EXAMPLES OF CLINICAL APPLICATIONS

The stages of differentiation of haematopoietic cells are characterised by the expression, or the non-expression, of surface antigens that can be identified using monoclonal antibodies with well defined specificity. One of the difficulties encountered during analysis of these antigens using flow cytometry, is the existence of a more or less marked non-specific fixation of monoclonal antibodies during staining. In order to ensure its specificity, it is necessary to take the effect of the non-specific fixation into account in the signal measured after staining (1, 2).
This IOTest 3 range antibody combination is used as a negative control for IOTest 3 combinations, including CD45-ECD. In fact, it serves to determine the non-specific staining of mixtures of antibodies conjugated with fluoresceine isothiocyanate (FITC) and R Phycoerythrin (PE) belonging to this range.
The distribution of negative populations should be similar to the background noise arising from autofluorescence and from non-specific staining for each of the populations of interest. A histogram of the diffusion of narrow angle light (Forward Scatter or FS) *versus* orthogonal diffusion of light (Side Scatter or SS) enables lymphocytes to be discriminated from debris. An acquisition window carried out on a histogram of the orthogonal diffusion of light (SS) *versus* fluorescence of CD45-ECD enables the population of interest to be isolated, which is then analysed on an FITC *versus* PE histogram.
The same acquisition window (obtained as SS *versus* CD45-ECD) is then again used for the analysis, making use of the specific mixture of conjugated IOTest3 antibodies used for the test.

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.
   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.

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.
Samples should be analysed within 24 hours of venipuncture.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for taking samples.
- Automatic pipettes with disposable tips to take 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).
- A combination of conjugated antibodies specific to the IOTest 3 reagent line the third constituent of which is CD45-ECD.
- 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 analysed, a suitable control tube is used along with a test tube. When the specific IOTest 3 conjugated antibody combination includes CD45-ECD as a third constituent one of the adapted negative controls is the Neg.Ctrl.-FITC / Neg.Ctrl.-PE / CD45-ECD combination (Ref. A07729).

1. Add 20 µL of specific IOTest 3 conjugated antibodies to each test tube, and 20 µL of the appropriate negative control to each control tube. (Ref. A07729).
2. Add 100 µL of the test sample to the 2 tubes. Vortex the tubes slowly.
3. Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4. Then perform, if necessary, 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. If the sample does not contain red cells, add 2 mL of PBS.
5. Centrifuge for 5 minutes at 300 x g at room temperature.
6. Remove the supernatant by aspiration.
7. Resuspend the cell pellet using 3 mL of PBS.
8. Repeat stage 5.
9. Remove the supernatant by aspiration and resuspend the cell pellet using:

– 0.5 mL or 1 mL of IOTest 3 Fixative Solution (Ref. A07800) at its working concentration

(1X), if the preparations are to be kept for more than 2 hours and for less than 24 hours,
- 0.5 mL or 1 mL of PBS without formaldehyde, if the preparations are to be analysed within 2 hours.

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

PERFORMANCE

SPECIFICITY

The 679.1Mc7 monoclonal antibody (mAb), belonging to the isotypic subclass IgG1, does not bind specifically to any of the differentiation antigens present on the surface of human leucocytes.

MAb J33 reacts with all the isoforms of the CD45 molecule (180 to 220 kDa); it is therefore referenced as a panleucocytic marker. MAb J33 was assigned to CD45 during the 3rd HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Oxford, England, in 1986 (WS Code: 818, Section NL) (3).

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 10 healthy adults were tested using the reagent described above. The mean values of the results obtained in the leucocyte sub-populations of interest are shown in the tables below:

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD45+	10	91.9	6.63	7

Monocytes	Number	Mean (%)	SD	CV (%)
CD45+	10	86.6	11.34	13

Granulocytes	Number	Mean (%)	SD	CV (%)
CD45+	10	99.7	0.30	0.3

INTRA-LABORATORY REPRODUCIBILITY

On the same day, using the same cytometer and for the same positive target (KG-1a line), 12 measurements of the percentage of CD45 positive cells and 12 measurements of the Mean of Fluorescence Intensity (MFI) of negative cells for the isotypic controls were performed. The results obtained are shown in the following table:

Cell Line KG-1a	Number	Mean	SD	CV (%)
MFI (IgG1-FITC)	12	1.78	0.03	1.83
MFI (IgG1-PE)	12	0.72	0.02	2.98
% CD45	12	99.9	0.07	0.07

INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same positive target (KG-1a line), 12 measurements of the percentage of CD45 positive cells and 12 measurements of the Mean of Fluorescence Intensity (MFI) of negative cells for the isotypic controls were performed by two technicians and the preparations were analysed on two different cytometers. The results obtained are shown in the following tables:

Cytometer n°1:

Cell Line KG-1a	Number	Mean	SD	CV (%)
MFI (IgG1-FITC)	12	1.78	0.03	1.8
MFI (IgG1-PE)	12	0.72	0.02	3.0
% CD45	12	99.9	0.07	0.1

Cytometer n°2:

Cell Line KG-1a	Number	Mean	SD	CV (%)
MFI (IgG1-FITC)	12	1.64	0.03	1.6
MFI (IgG1-PE)	12	0.52	0.02	3.2
% CD45	12	99.98	0.09	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. 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 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/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 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 labelling.
7. CD45-negative or very weakly-positive acute lymphoblastic leukaemia have been described. For these, the lymphocytic origin of the blast cells should be confirmed using other markers.

MISCELLANEOUS

See the Appendix for examples and references.

TRADEMARKS

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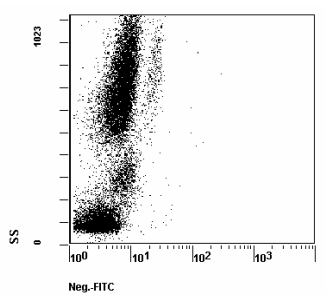
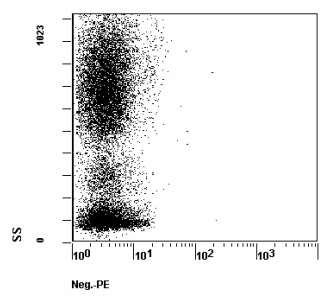
APPENDIX TO REF A07729

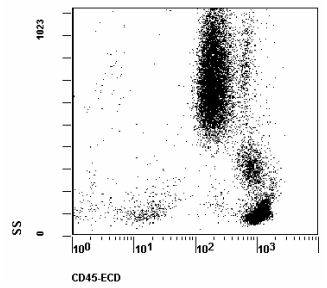
EXAMPLES

The 3 diagrams below are biparametric representations (Fluorescence Intensity *versus* Side Scatter) of a normal peripheral whole blood specimen. Staining is with Negative-FITC / Negative-PE / CD45-ECD Conjugated Antibodies (Ref. A07729). Lysis and fixation are with IOTest 3 Lysing Solution (Ref. A07799) and IOTest 3 Fixative Solution (Ref. A07800) respectively. All events acquired are shown in all representations.

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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