



**IOTest<sup>®</sup> 3**  
Conjugated Antibodies

**PN IM3481**

**25 tests**  
**20 µL/test**

**CD14-FITC**  
**CD13-PE**  
**CD45-ECD**

**For Research Use Only. Not For Use In Diagnostic Procedures.**

**REAGENT**

IOTest 3 Conjugated Antibodies – CD14-FITC / CD13-PE / CD45-ECD  
PN IM3481 – 25 tests – 20 µL/test

	CLONE 1	CLONE 2	CLONE 3
Specificity	CD14	CD13	CD45
Clone	RMO52	SJ1D1	J33
Hybridoma	SP2/0 x Balb/c	SP2/0 x Balb/c	NS1 x Balb/c
Immunogen	Isolated human monocytes	KG-1 cell line	Laz 221 ALL cell line
Ig Chain	IgG2a	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites fluid	Ascites fluid	Ascites fluid
Purification	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography
Buffer	2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.		
Conjugation	Fluorescein isothiocyanate (FITC)	PE (R-Phycoerythrin)	ECD™ (Phycoerythrin-Texas-Red®-X)

**SPECIFICITY**

The CD14 molecule is found on cells of myelomonocytic lineage. It is strongly expressed on monocytes, macrophages, and weakly on neutrophils (1, 2). It is also present on pleural phagocytic cells and on reticular dendritic cells, on Langerhans cells, and histiocytes (3, 4). CD14 is not expressed on B lymphocytes, T lymphocytes, NK cells, red blood cells and platelets.

The RMO52 monoclonal antibody (mAb) does not react with T or B lymphocytes (1, 2). It has been assigned to the CD14 cluster of differentiation during the 6th International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Kobe, Japan, in 1996 (WS Code: MA62, Section M) (5).

The CD13 molecule, also known as aminopeptidase N (APN), is expressed early in the myelo-monocytic lineage (i.e. monocytes, neutrophils, eosinophils and basophils) and in their respective committed progenitors (i.e. granulocyte-macrophage colony forming unit) (6–9). The CD13 antigen is expressed on cells from non-hematopoietic tissues, such as epithelial cells from renal proximal tubules and from intestinal brush border, endothelial cells, fibroblasts, brain cells, bone marrow stromal cells, osteoclasts and cells lining the biliary caniculaea (10).

The SJ1D1 mAb reacts in peripheral blood samples with monocytes, neutrophils, eosinophils, and basophils. It has been clustered as CD13 during the 3rd International HLDA Workshop, in Oxford, England, in 1986 (WS Code: 285, Section M) (7).

The CD45 molecules comprise five different isoforms generated by alternative splicing of three exons encoding peptide segments designated A, B and C (11). Antibodies that belong to the CD45 cluster recognize all CD45 isoforms. CD45 molecule is expressed on the surface of all human leucocytes.

The J33 mAb binds to all the CD45 isoforms present on human leucocytes.

It has been assigned to the CD45 cluster of differentiation at the 3rd International HLDA Workshop in Oxford, England, in 1986 (WS Code: 818, Section NL) (12).

**CONJUGATION**

Fluorescein isothiocyanate (FITC) is conjugated at 6 – 10 moles of FITC per mole of Ig. Excitation wavelength: 488 nm  
Maximum emission wavelength: 525 nm  
Main emission color: Green

R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig. Excitation wavelength: 488 nm  
Maximum emission wavelength: 575 nm  
Main emission color: Orange-red

R-phycoerythrin covalently linked to Texas Red (PE-TxR or ECD) is conjugated at 0.5 – 1.5 moles of ECD per mole of Ig.

Excitation wavelength: 488 nm  
Maximum emission wavelength: 613 nm  
Main emission color: Red

**APPLICATION**

Multiparametric flow cytometry analysis of CD14, CD13 and CD45 antigen expression in hematopoietic myeloid neoplasia.

Immunophenotyping of acute leukemias using CD45 antigen to gate blasts on a side scatter versus CD45 representation (13 – 15).

Characterization and differentiation between AML (Acute Myeloblastic Leukemia) with monocytic differentiation (CD14<sup>+</sup>CD13<sup>+</sup>CD45<sup>dim</sup>) and AML without monocytic origin (CD14<sup>-</sup>CD13<sup>+</sup>CD45<sup>dim</sup>) (13, 14, 16).

**STATEMENT OF WARNINGS**

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

**STORAGE CONDITIONS AND STABILITY**

Each reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

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**BECKMAN  
COULTER™**

**PN IM3481****25 tests****20 µL/test****CD14-FITC****CD13-PE****CD45-ECD****For Research Use Only. Not For Use In Diagnostic Procedures.****REAGENT PREPARATION**

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

**PROCEDURE**

This reagent is designed for flow cytometry.

Assay volume: 20 µL per  $5 \times 10^5$  cells in one test, or per 100 µL whole blood or bone marrow.

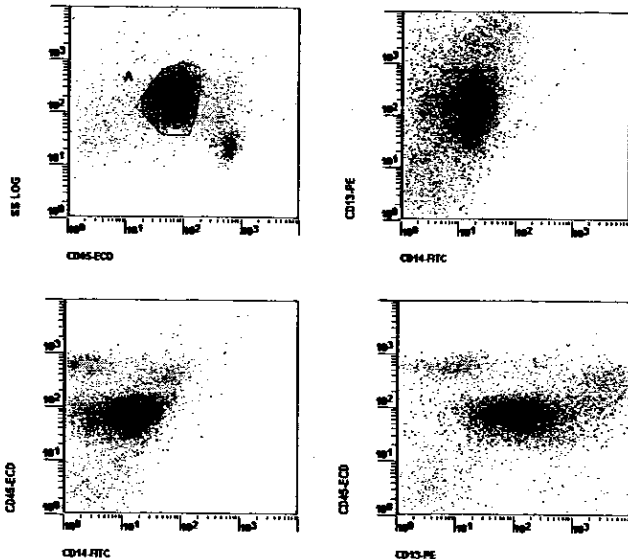
A wash is required to yield optimal results.

The use of IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) procedures are recommended to yield optimal results.

**EXAMPLE DATA**

The 4 diagrams below are biparametric representations (Side Scatter versus Fluorescence Intensity or Fluorescence Intensity versus Fluorescence Intensity) of an Acute Myeloblastic Leukemia specimen (AML-M3, Bone marrow aspirate). Staining is with CD14-FITC / CD13-PE / CD45-ECD Conjugated Antibodies (PN IM3481). Lysis and fixation are with IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) respectively. All events acquired are shown. Region A defines the gating strategy (CD45 positive blasts cluster) 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 the EXPO™ Cytometer Software (PN 6605434).

**SELECTED RESEARCH REFERENCES**

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**MISCELLANEOUS**

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