

**PN IM3475****25 tests****20 µL/test****CD71-FITC****CD33-PE****CD45-ECD**
**IO Test<sup>®</sup> 3**  
 Conjugated Antibodies

**For Research Use Only. Not For Use In Diagnostic Procedures.**
**REAGENT**
 IO Test 3 Conjugated Antibodies – CD71-FITC / CD33-PE / CD45-ECD  
 PN IM3475 – 25 tests – 20 µL/test

	CLONE 1	CLONE 2	CLONE 3
Specificity	CD71	CD33	CD45
Clone	YDJ1.2.2	D3HL60.251	J33
Hybridoma	X63-Ag8.653 x Balb/c	NS1 x Balb/c	NS1 x Balb/c
Immunogen	MLA 144 (Gibbon leukemic cell line)	HL60 cell line	Laz 221 ALL cell line
Ig Chain	IgG1	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	Fluoresceine isothiocyanate (FITC)	PE (R-Phycoerythrin)	ECD™ (Phycoerythrin-Texas-Red®-X)

**SPECIFICITY**

The CD71 molecule, known as the transferrin receptor or T9 antigen is expressed by reticulocytes, erythroid precursors and capillary endothelial cells in brain (1). All other known cell types express CD71 only when entering in proliferation (1).

The YDJ1.2.2 monoclonal antibody (mAb) has been assigned to the CD71 cluster of differentiation at the 5th International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Boston, U.S.A., in 1993 (WS Code: A006 and PB0111, Section A and PB respectively) (2).

The CD33 differentiation antigen is expressed by hematopoietic progenitor cells on colony-forming units for granulocytes, erythrocytes, monocytes and megakaryocytes (CFU-GEMM) (3). It is also present on progenitors of granulocytes and mononuclear phagocytes (CFU-GM) and on early erythroid progenitors (BFU-E) (3).

The D3HL60.251 mAb reacts with cells of myeloid origin, strongly on monocytes, and weakly on granulocytes of the peripheral blood. It does not react with mature lymphoid cells or lymphoid precursors. The D3HL60.251 mAb has been assigned to the CD33 cluster of differentiation at the 4th International HLDA Workshop in Vienna, Austria, in 1989 (WS Code: 504, Section M) (4).

The CD45 molecules comprise five different isoforms generated by alternative splicing of three exons encoding peptide segments designated A, B and C (5). 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) (6).

**CONJUGATION**

Fluorescein isothiocyanate (FITC) is conjugated at 5 – 9 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 CD71, CD33 and CD45 antigen expression in hematopoietic myeloid and erythroid neoplasia.

Immunophenotyping of acute leukemias using CD45 antigen to gate blasts on a side scatter versus CD45 representation (7 – 9).

Characterization and differentiation of blasts with myeloid lineage differentiation (CD71<sup>+</sup>CD33<sup>+</sup>) from blasts with erythroid lineage origin (CD71<sup>+</sup>CD33<sup>-</sup>) (7, 8, 10).

**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.

**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.

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**BECKMAN  
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**PN IM3475****25 tests****20 µL/test****CD71-FITC****CD33-PE****CD45-ECD****For Research Use Only. Not For Use In Diagnostic Procedures.****PROCEDURE**

This reagent is designed for flow cytometry.

Assay volume: 20 µL per 5 x 10<sup>5</sup> cells in one test, or per 100 µL whole blood or bone marrow.

A wash is required to yield optimal results.

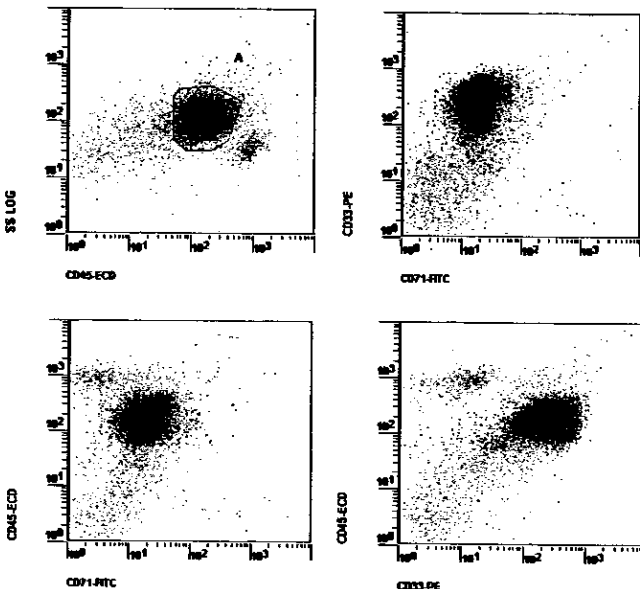
The use of IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) procedures are recommended to yield optimal results.

**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 (bone marrow aspirate) (AML-M5) specimen. Staining is with CD71-FITC/CD33-PE/CD45-ECD Conjugated Antibodies (PN IM3475). Lysis and fixation are with IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) respectively. All events acquired are shown. The blasts are shown in dark in all histograms. Region A defines the gating strategy (CD45 positive cluster) used on this example.

Acquisition is with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software. Analysis is with the EXPO™ Cytometer software (PN 6605434).

Example: Acute Myeloblastic Leukemia (AML-M5).

**SELECTED RESEARCH REFERENCES**

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**MISCELLANEOUS**

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