

PN IM3469**25 tests****20 µL/test****CD5-FITC****CD23-PE****CD19-ECD**
IO Test[®] 3
 Conjugated Antibodies

For Research Use Only. Not For Use In Diagnostic Procedures.
REAGENT
 IO Test 3 Conjugated Antibodies – CD5-FITC / CD23-PE / CD19-ECD
 PN IM3469 – 25 tests – 20 µL/test

	CLONE 1	CLONE 2	CLONE 3
Specificity	CD5	CD23	CD19
Clone	BL1a	9P25	J4.119
Hybridoma	SP2/0-Ag14 x Balb/c	NS1 x Balb/c	NS1 x Balb/c
Immunogen	Human thoracic duct lymphocytes	EBV transformed lymphoblastoid cell line	SKLY 18 lymphoma cells
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	Fluoresceine isothiocyanate (FITC)	PE (R-Phycoerythrin)	ECD™ (Phycoerythrin-Texas-Red®-X)

SPECIFICITY

The CD5 molecule is expressed on mature T lymphocytes, on most thymocytes and is also present on a subpopulation of B lymphocytes (1–3). It is not expressed on granulocytes, monocytes or platelets (3).

The BL1a monoclonal antibody (mAb) has been assigned to the CD5 cluster of differentiation during the 3rd International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Oxford, England, in 1986 (WS Code: 520, Section T) (1, 2).

The CD23 antigen is primarily expressed on B lymphocytes and monocytes. It is also present on a large variety of other cells such as T lymphocytes, eosinophils, platelets, Langerhans cells, a subset of thymic epithelial cells and neutrophils. On B lymphocytes, CD23 expression is upregulated upon activation and ultimately lost upon plasmocyte differentiation.

The 9P25 mAb was assigned to the CD23 cluster of differentiation at the 6th International HLDA Workshop in Kobe, Japan, in 1996 (WS Code: CD23.1, Section B) (4, 5).

The CD19 molecule shows a full B lineage expression, from early pre-B cells to mature B lymphocytes. Its expression is lost during maturation to plasma cells (6–8). CD19 expression is not observed in normal T lymphocytes, NK cells, monocytes, and granulocytes (8). The J4.119 mAb was assigned to the CD19 cluster of differentiation at the 4th International HLDA Workshop in Oxford, England, in 1986 (WS Code: B191, section B) (9).

CONJUGATION

Fluorescein isothiocyanate (FITC) is conjugated at 3–10 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 CD5, CD23 and CD19 antigen expression in hematopoietic lymphoid neoplasia.

Identification and differentiation of the B-cells neoplasia coexpressing CD5 and CD19 from CD5⁺ T lymphoproliferative disorders (10, 11).

Characterization and differentiation between CD5 expressing B-neoplasia: i.e. B-CLL/SLL (B- Chronic lymphocytic Leukemia / Small Lymphocytic Lymphoma) which are CD5⁺CD23⁺CD19⁺ and MCL (Mantle Cell Lymphoma) with CD5⁺CD23⁻CD19⁺ phenotype. (10–15).

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.

PROCEDURE

This reagent is designed for flow cytometry.

Assay volume: 20 µL per 5 x 10⁵ cells in one test, or per 100 µL whole blood or bone marrow.

A wash is required to yield optimal results.

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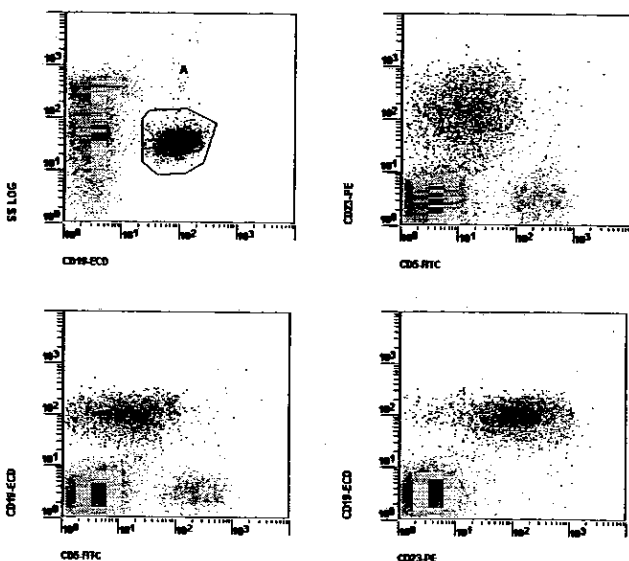
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The use of IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) procedures are recommended to yield optimal results.

EXAMPLE DATA

The 4 diagrams below are biparametric representations (Side Scatter versus Fluorescence Intensity or Fluorescence Intensity versus Fluorescence Intensity) of a B-cell Chronic Lymphocytic Leukemia specimen (B-CLL, Peripheral blood). Staining is with CD5-FITC / CD23-PE / CD19-ECD Conjugated Antibodies (PN IM3469). 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 (CD19 positive 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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Manufactured by:
Immunotech, a Beckman Coulter Company
130, avenue de Lattre de Tassigny, B.P. 177
13276 Marseille Cedex 9, France

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3469EX070600 Vers. 02/ 07/06/00 AC-00-0626

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Manufactured by Immunotech, a Beckman Coulter Company.
130, avenue de Lattre de Tassigny B.P. 177, 13276 Marseille Cedex 9, France
Tel: (33) 4 91 17 27 00 - Fax: (33) 4 91 41 43 58
e-mail: abmarket@immunotech.fr - www.beckmancoulter.com

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