

PN IM3489**25 tests****20 µL/test****Negative-FITC****Negative-PE****CD45-ECD**
IO Test[®] 3
 Conjugated Antibodies

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

IO Test 3 Conjugated Antibodies – Negative-FITC / Negative-PE / CD45-ECD

PN IM3489 – 25 tests – 20 µL/test

| | CLONE 1 | CLONE 2 | CLONE 3 |
|--------------|---|---|--|
| Specificity | N/A | N/A | CD45 |
| Clone | 679.1Mc7 | 679.1Mc7 | J33 |
| Hybridoma | 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 | Lazz 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 | Fluorescein isothiocyanate (FITC) | PE (R-Phycoerythrin) | ECD [™] (Phycoerythrin-Texas-Red [®] -X) |

N/A = not applicable

SPECIFICITY

Hematopoietic cell differentiation is characterized by the expression of distinct membrane antigens at specific stages of the cellular maturation. These antigens expressed on the membrane are identified by specific monoclonal antibodies.

Certain monoclonal antibodies have irrelevant specificities: they induce nonspecific immunolabeling on hematopoietic cells and platelets (1).

The 679.1Mc7 monoclonal antibody shares certain structural characteristics (i.e. isotypes and conjugated fluorochromes) with the monoclonal antibodies of interest (i.e. specific of hematopoietic cell surface antigens) but is devoid of any relevant specificities with regard to the studied cell population (1).

The CD45 molecules comprise five different isoforms generated by alternative splicing of three exons encoding peptide segments designated A, B and C (2). 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 Workshop on Human Leucocyte Differentiation Antigens in Oxford, England, in 1986 (WS Code: 818, Section NL) (3).

CONJUGATION

Fluorescein isothiocyanate (FITC) is conjugated at 2 – 5 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

Negative control for multiparametric flow cytometry analysis of leucocytes antigens expression using FITC / PE / ECD as fluorochromes and CD45-ECD for blasts gating (4 – 6).

The negative staining pattern related to each analyzed fluorescences (i.e. FITC, PE, and ECD) is designed to match the level of background fluorescence resulting from autofluorescence and nonspecific binding for each specific CD45⁺ gated population (4).

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.

3489EX230600 Vers. 02/ 23/06/00 AC-00-0712

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**BECKMAN
COULTER[™]**

PN IM3489

25 tests

20 µL/test

**Negative-FITC /
Negative-PE /
CD45-ECD**

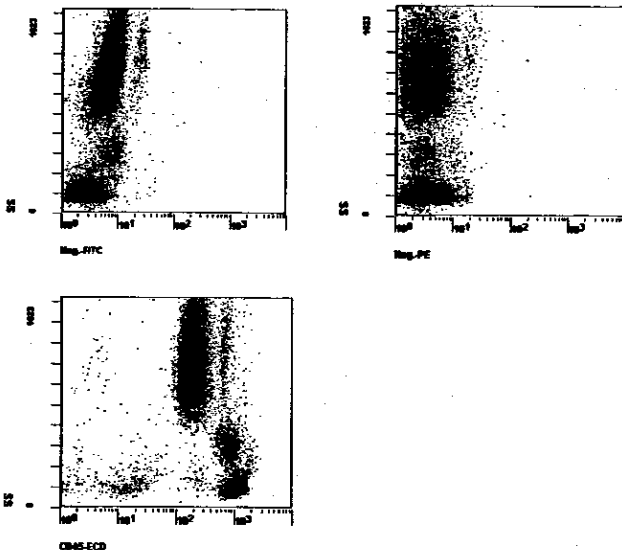
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The use of IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) procedures are recommended to yield optimal results.

EXAMPLE DATA

The 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 (PN IM3489). Lysis and fixation are with IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) 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 the EXPO™ Cytometer Software (PN 6605434).



SELECTED RESEARCH REFERENCES

- [2951] Stewart, C.C., Stewart, S.J., "Cell preparation for the identification of leukocytes", 1994, *Methods Cell Biol.*, Chap3, 41, 39-60.
- [285] Serra-Pages, C., Morimoto, C., Schlossman, S.F., Saito, H., Streuli, M., "Characterization of CD45 mAb", 1995, *Leucocyte Typing V, White Cell Differentiation Antigens*. Schlossman, S.F., et al., Eds., Oxford University Press, 389-391.
- [286] Cobbold, S., Hale, G., Waldmann, H., "Non-lineage, LFA-1 family, and leukocyte common antigens: New and previously defined clusters", 1987, *Leucocyte Typing III, White Cell Differentiation Antigens*, McMichael A.J., et al., Eds., Oxford University Press, 788-803.
- [5483] Borowitz, M., Bauer, K.D., Duque, R.E., Horton, A.F., Marti, G., Muirhead, K.A., Peiper, S., Flickman, W., "Clinical applications of flow cytometry: Quality assurance and immunophenotyping of lymphocytes; approved guideline", 1998, *NCCLS*, 21, 18.
- [5134] Seltzer, G.T., Shults, K.E., Loken, M.R., "CD45 gating for routine flow cytometric analysis of human bone marrow specimens", 1993, *Acad. Sciences*, 265-280.
- [272] Borowitz, M.J., Guenther, K.L., Shults, K.E., Stetler, G.T., "Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis", 1993, *Am. J. Clin. Pathol.*, 5, 100, 534-540.

MISCELLANEOUS

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