

**PN IM3488****25 tests  
20 µL/test****Negative-FITC  
Negative-PE  
Negative- ECD****IOTest<sup>®</sup> 3**  
Conjugated Antibodies**For Research Use Only. Not For Use In Diagnostic Procedures.****REAGENT**IOTest 3 Conjugated Antibodies – Negative-FITC / Negative-PE / Negative-ECD  
PN IM3488 – 25 tests – 20 µL/test

	CLONE 1	CLONE 2	CLONE 3
Specificity	N/A	N/A	N/A
Clone	679.1Mc7	679.1Mc7	679.1Mc7
Hybridoma	P3-X63-Ag.8.653 x Balb/c	P3-X63-Ag.8.653 x Balb/c	P3-X63-Ag.8.653 x Balb/c
Immunogen	Non-biological hapten	Non-biological hapten	Non-biological hapten
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).

**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.

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 non-specific binding of each specific conjugated antibody staining (2, 3).

**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 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 9 diagrams next page are biparametric contour dot plot representations (Fluorescence Intensity versus Fluorescence Intensity) of a normal peripheral whole blood specimen.

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**BECKMAN  
COULTER**

# PN IM3488

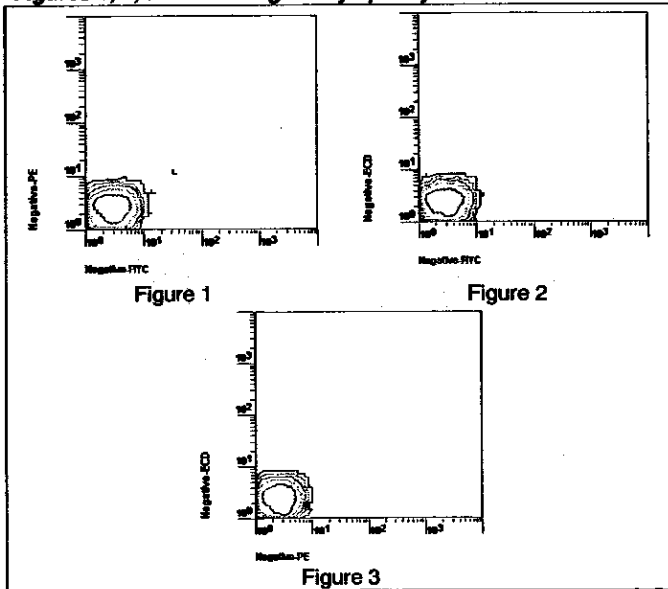
25 tests  
20 µL/test

Negative-FITC  
Negative-PE  
Negative-ECD

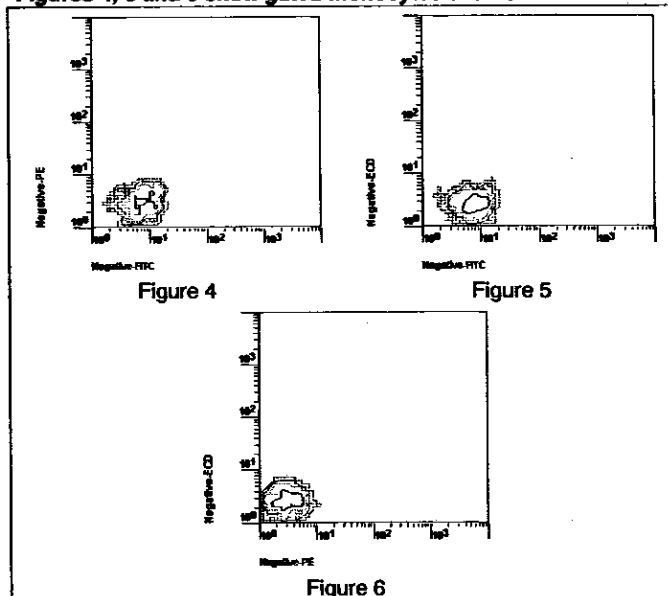
For Research Use Only. Not For Use In Diagnostic Procedures.

Staining is with Negative-FITC / Negative-PE / Negative-ECD Conjugated Antibodies (PN IM3488). Lysis and fixation are with IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) respectively. Studied events are gated on a size versus structure diagram. Acquisition is with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ Software. Analysis is with the EXPO™ Cytometer Software (PN 6605434).

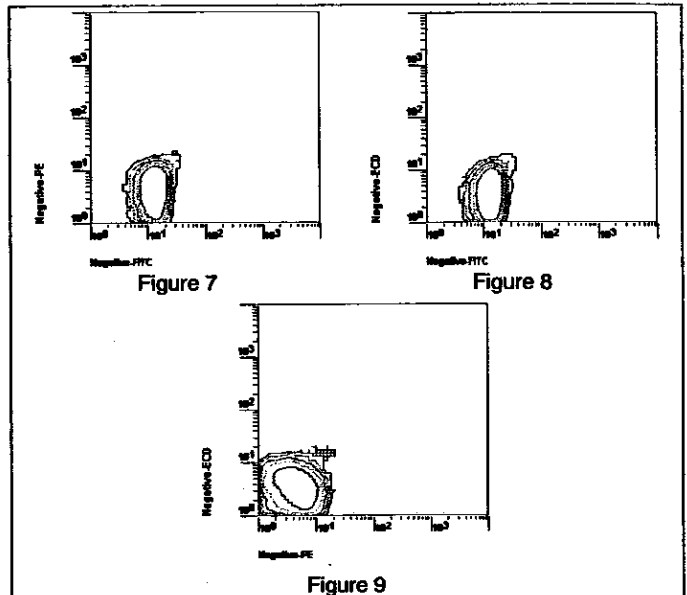
Figures 1, 2, and 3 show gated lymphocytes events.



Figures 4, 5 and 6 show gated monocytes events.



Figures 7, 8, and 9 show gated granulocytes events.



## 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.
- [5483] Borowitz, M., Bauer, K.D., Duque, R.E., Horton, A.F., Marti, G., Muirhead, K.A., Peiper, S., Rickman, W., "Clinical applications of flow cytometry: Quality assurance and immunophenotyping of lymphocytes; approved guideline", 1998, NCCLS, 21, 18.
- [173] Rothe, G., Schmitz, G., Adorf, D., Barlage, S., Gramatzki, M., Höffkes, H.G., Janossy, G., Knüchel, R., Ludwig, W.D., Nebe, T., Nerl, C., Orfao, A., Serke, S., Sonnen, R., Tichelli, A., Wörmann, B., "Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies", 1996, Leukemia, 10, 877-895.

## MISCELLANEOUS

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