

## ISOTYPIC CONTROL

## IgG1 (mouse)

Cat. No.	Form	Quantity	Presentation
0571	Purified	100 tests	Liquid 2 mL
0639	FITC	100 tests	Liquid 2 mL
0670	PE	100 tests	Liquid 2 mL
1473	PE-Cy5	100 tests	Liquid 2 mL
2475	APC	100 tests	Liquid 1 mL

**Warning**

*APC-conjugated forms of the IOTest® line of reagents are to be used at 10 µL / test instead of 20 µL / test.*

**Clone**

679.1Mc7

**Isotype**

IgG1 (mouse)

**Immunogen**

Non biological hapten

**Hybridoma**

Myeloma X63 Ag.8 x Balb/c spleen cells

**Specificity**

The 679.1Mc7 monoclonal antibody has an irrelevant specificity. It induces nonspecific binding on platelets and leucocyte membranes.

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 monoclonal antibodies (1).

When using a specific monoclonal antibody for a flow cytometric analysis the background must be taken into consideration. This background constitutes the nonspecific component of the labeling and should be distinguished from positive signal (2).

In order to analyze correctly the specific immunolabeling of biological samples by flow cytometry, it is necessary to check the proportion of nonspecific labeling. This can be done with an antibody sharing certain structural characteristics (isotype and conjugated fluorochrome) with the monoclonal antibody of interest, but being devoided of any relevant specificities with regard to the studied cell population.

**Applications**

The 679.1Mc7 monoclonal antibody is an isotypic control for flow cytometric setting before the use of unconjugated or conjugated specific mouse monoclonal antibodies of the IgG1 isotype. These formulations are optimized for cell surface immunolabeling studies.

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MA003

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES

**IMMUNOTECH**

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- Buffer** 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.
- Conjugation**
- FITC:** Fluorescein isothiocyanate (FITC) is conjugated at 2-6 moles of FITC per mole of IgG.  
Excitation wavelength: 488 nm  
Maximum emission wavelength: 525 nm  
Main emission color: Green
- PE:** R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of IgG.  
Excitation wavelength: 488 nm  
Maximum emission wavelength: 575 nm  
Main emission color: Orange-red
- PE-Cy5:** The IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanin 5.1 at 0.7-1 mole of PE-Cy5 per mole of IgG.  
Excitation wavelength: 488 nm  
Maximum emission wavelength: 670 nm  
Main emission color: Deep-red
- APC:** Allophycocyanin (APC) is conjugated at 0.7-1 mole of APC per mole of IgG.  
Excitation wavelength: 633-635 nm  
Maximum emission wavelength: 660 nm  
Main emission color: Deep-red

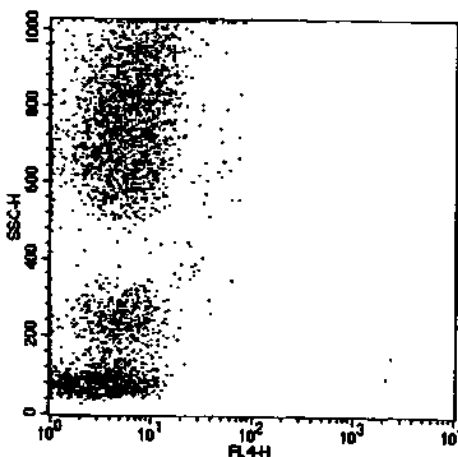
**Limitation:** APC conjugates are recommended for use only on flow cytometers equipped with an exciting source of 633 nm (He-Ne laser) or 635 nm (Red diode laser).

- Storage**
- The purified liquid form should be stored at 2-8°C until the expiration date stated on the vial label.
- The conjugated forms should not be frozen and should be stored in the dark at 2-8°C until the expiration date stated on the vial label.

- Recommended Procedures**
- Flow cytometry
- Purified, FITC-, PE- and PE-Cy5-conjugated forms: 20  $\mu$ L /  $5 \times 10^5$  cells or 100  $\mu$ L whole blood.
- APC-conjugated form: A specific calibration is applied to facilitate the blending of conjugated antibodies in multiparametric flow cytometry.  
10  $\mu$ L /  $5 \times 10^5$  cells or 100  $\mu$ L whole blood.
- Limitation:** R-phycoerythrin (PE) is sensitive to light exposure. Consequently, PE- or PE-Cy5-conjugated antibodies are not suitable for fluorescence microscopy.

**Results  
Example**

The graph below is a double parameter representation (Side Scatter *versus* Fluorescence 4) of a lysed whole blood sample from a healthy donor. Staining is with IgG1-APC (Cat. No. 2475). Along the Y axis, lymphocytes are events with low side scatter values, monocytes show low to medium side scatter values and neutrophils show medium to high side scatter values.



Analysis is with a Becton Dickinson FACSCalibur™ flow cytometer equipped with CELLQuest™ software.

IOTest is a registered trademark of Immunotech, a Coulter Company. FACSCalibur and CELLQuest are trademarks of Becton Dickinson Immunocytometry Systems (BDIS).

**References**

- 1) Lanier, L.L., Recktenvald, D.J., "Multicolor immunofluorescence and flow cytometry", 1989, in Methods: A Companion to Methods in Enzymology, 2, 192.
- 2) Centers for Disease Control and Prevention: 1994 revised guidelines for performance of CD4<sup>+</sup> T cell determinations in persons with human immunodeficiency virus (HIV) infection, Mortality and Morbidity Weekly Report, 43, 3.

