



Marseille, January 5, 2004

Subject: Replacement of Mouse IgG2a Isotypic Control Clone **U7.27**
by Clone **7T4-1F5**

Dear Customer,

Beckman Coulter, Inc. thanks you for your continued support of its products and would like to provide you with information regarding the product you have just received and that contains an isotypic control, **mouse IgG2a**, alone or in addition to another isotypic control.

You will note that we have changed the IgG2a-producing clone because of occasional non-specific binding of the **PE-conjugated form** of former clone **U7.27 (PN IM0671)** to the ECD tandem dye. So we have re-developed the entire series of conjugated mouse IgG2a isotypic controls with the **7T4-1F5** clone, another mouse IgG2a-producing clone; its PE-conjugated form does not show this defect.

The table below gives you the new part numbers that have been created to reflect this change.

Product Name	Status	Old Part Numbers (with clone U7.27)	New Part Numbers (with clone 7T4-1F5)
IgG2a-FITC	RUO	IM0640	A12689
	ASR	IM0640	A12690
IgG2a-PE	RUO	IM0671	A09143
	ASR	IM0671	A09141
	CE-marked	Not applicable	A09142
IgG2a-ECD	RUO	IM2715	A09146
	ASR	IM2715	A09144
	CE-marked	Not applicable	A09145
IgG2a-PC5	RUO	IM2664	A09149
	ASR	IM2664	A09147
	CE-marked	Not applicable	A09148

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IgG2a-PC7	RUO	Not applicable	A12691
IgG2a-APC	RUO	IM2476	A12693
	ASR	Not applicable	A12694
IgG1-FITC/IgG2a-PE	RUO	IM1204	A10973
	ASR	IM1204	A10974
	AFSSAPS	IM1204	Not applicable
	CE-marked	Not applicable	A10975
IgG2a-FITC/IgG2a-PE	RUO	IM1208	A12695
Isotypic Control IgG(1+2a)-PE included in the CD34 Pool Kit (PN IM1459 or IM1459U)	RUO	IM1647	A10976*
	ASR	IM1647U	A10977*

* Not available separately

The new products perform and are to be used just like the old ones (for more information, please read the package insert).

We apologize for any inconvenience that those changes may cause.

If you have any questions or concerns regarding those new products, please do not hesitate to contact me or your local Beckman Coulter representative.

Sincerely,



Michel PAGNIEZ, Pharm.
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Cellular Analysis, Systems Biology
Immunotech, a Beckman Coulter Company
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AC-03-1236

IOTest[®] Mouse IgG2a-APC Isotypic Control

PN A12693 – 100 tests – 10 µL/test – Clone 7T4-1F5

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

SPECIFICITY

The 7T4-1F5 monoclonal antibody has an irrelevant specificity. It induces nonspecific immunolabeling on hematopoietic cells and therefore constitutes an isotype control.

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 the specific immunolabeling of biological samples by flow cytometry, it is necessary to check the proportion of nonspecific labeling. This can be done by using an isotype control. The isotype control shares certain structural characteristics (isotype and conjugated fluorochrome) with the monoclonal antibody of interest, but is devoid of any relevant specificities with regard to the studied cell population (3).

REAGENT

IOTest Mouse IgG2a-APC Isotypic Control
PN A12693 – 100tests – 10 µL/test

Clone 7T4-1F5

Isotype IgG2a

Immunogen A totally unique clonotypic determinant present on the cell line used for immunization

Hybridoma NS1 x Balb/c

Source Ascites fluid

Purification Protein A chromatography

Conjugation Allophycocyanin (APC) is conjugated at 0.5 – 1.5 moles of APC per mole of Ig.

Excitation wavelength: 633-635 nm

Maximum emission wavelength: 660 nm

Main emission color: Deep-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

This reagent is an isotypic control for mouse APC-conjugated monoclonal antibodies (mAb) of the IgG2a isotype. It may be used to monitor levels of non-specific antibody

binding and adjust flow cytometer APC-settings in cell surface staining procedures that use APC-conjugated mAb reagents of the mouse IgG2a subclass.

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

This 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

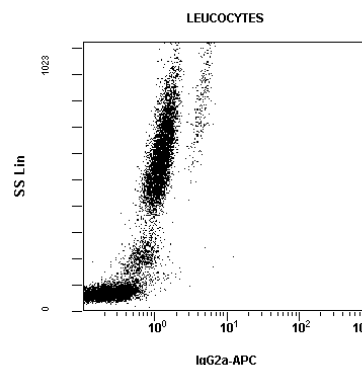
This reagent is designed for Flow Cytometry. Assay volume: 10 µL per 5 x 10⁵ cells in one test, or per 100 µL whole blood. A wash is required to yield optimal results.

EXAMPLE DATA

The histogram below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of lyzed normal whole blood sample. Staining is with IgG2a-APC isotypic control (PN A12693).

Acquisition is with a BECKMAN COULTER[®] CYTOMICS[™] FC 500 flow cytometer.

Analysis is with the CYTOMICS RXP[™] analysis software.



SELECTED RESEARCH REFERENCES

1. Lanier, L.L., Reckenwald, D.J., "Multicolor immunofluorescence and flow cytometry", 1991, Methods (in Enzymology), 3, 2, 192-199.
2. MMWR, "1997 revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus (HIV)", 1997, INIST CNRS, RR-2, 46, 1-29.
3. Stewart, C.C., Stewart, S.J., "Cell preparation for the identification of leukocytes", 1994, Methods Cell Biol., Chap3, 41, 39-60.

PRODUCT AVAILABILITY

IOTest Mouse IgG2a-APC Isotypic Control
PN A12693 – 100 tests – 10 µL/test

For additional information in the USA, call 800-526-7694.

Outside the USA, contact your local Beckman Coulter representative.

TRADEMARKS

IOTest is a registered trademark of Immunotech S.A.

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