

**Analyte Specific Reagent.**

Analytical and performance characteristics are not established.

**SPECIFICITY**

The CD69 molecule is a type II integral membrane protein with a molecular weight of 27 – 33 kDa under reduced conditions. It is expressed as a disulfide linked homodimer composed of differentially glycosylated subunits within an extracellular C-type lectin domain (1).

CD69 is the earliest inducible cell surface glycoprotein to appear upon *in vitro* activation of T cells, NK cells, and B cells (2). CD69 is undetectable on most of the circulating peripheral blood lymphocytes (PBL) (3). Resting T cells do not express CD69. However, they may express it rapidly by triggering of their TCR / CD3 complex. Similarly, the majority of peripheral blood NK cells are negative for CD69, but express it shortly after activation with PMA, IL-2, Interferon  $\alpha$ , or CD16 monoclonal antibody (4).

CD69 is constitutively expressed in subpopulations of thymocytes and platelets (5).

The TP1.55.3 monoclonal antibody reacts with activated T lymphocytes (3). It has been studied at the 4th International Workshop on Human Leucocyte Differentiation Antigen in Vienna, Austria, in 1989 (6)

**REAGENT**

IOTest CD69-PE Conjugated Antibody

PN IM1943U – 2 mL Liquid – 20 µL / test\*.

**Clone**

TP1.55.3

**Isotype**

IgG2b, mouse

**Immunogen**

Human PBL activated for 24h with PMA and CD3 mAb

**Hybridoma Source**

P3 X63-Ag8.653 x Balb/c Ascites fluid

**Purification**

Ion exchange or affinity chromatography

**Conjugation**

R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig.

**Fluorescence**

PE (orange-red)  
Excites at 486 – 580 nm  
Emits at 568 – 590 nm

**REAGENT CONTENTS**

This reagent is provided in phosphate-buffered saline, with 0.1% sodium azide (NaN<sub>3</sub>) as preservative, and 2.0 mg / mL bovine serum albumin (BSA).

**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. Do not use antibody beyond the expiration date on the label.
3. Samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
4. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
5. Minimize exposure of reagent to light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.

**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.

**EVIDENCE OF DETERIORATION**

Any change in the physical appearance of this PE-labeled reagent (clear colorless to pinkish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

**REAGENT PREPARATION**

No preparation is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

**SELECTED RESEARCH REFERENCES**

1. Lopez-Cabrera, M., Santis, A.G., Fernandez-Ruiz, E., Sanchez-Mateos, P., Sanchez-Madrid, F., "The human earliest lymphocyte activation antigen AIM / CD69 is a new member of the C-type animal lectin superfamily", 1995, Leucocyte Typing V, White Cell Differentiation Antigens. Schlossman, S.F., et al., Eds., Oxford University Press, 1126-1129.
2. Testi, R., d'Ambrosio, D., de Maria, R., Santoni, A., "The CD69 receptor: A multipurpose cell surface trigger for hematopoietic cells", 1994, Immunol. Today, 15, 479-483.
3. Cebrian, M., Yagüe, E., Rincon, M., Lopez-Botet, M., de Landazuri, M.O., Sanchez-Madrid, F., "Triggering of T-cell

proliferation through AIM, an activation inducer molecule expressed on activated human lymphocytes", 1988, J. Exp. Med, 168, 1621-1637.

4. Borrego, F., Galiani, M.D., Garcia-Cozar, F., Madueno, J.A., Perez-Bermejo, L., Santamaria, M., Pena, J., Solena, R., "CD69 expression and function on NK cells", 1995, Leucocyte Typing V, White Cell Differentiation Antigens. Schlossman, S.F., et al., Eds., Oxford University Press, 1427-1430.
5. Testi, R., Pulcinelli, F., Frati, L., Gazzaniga, P.P., Santoni, A., "CD69 is expressed on platelets and mediates platelet activation and aggregation", 1990, J. Exp. Med., 172, 701-707.
6. Cebrian, M., Sanchez-Mateos, P., Redondo, J.M., Ursa, A., De Landazuri, M.O., Sanchez-Madrid, F., "CD69 : A GP33/27 kDa activation inducer molecule (AIM) recognized by a group of mAb of the workshop activation panel. Induction of T-cell proliferation through the AIM activation antigen", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens. W. Knapp, et al., Eds., Oxford University Press, 441-444.

**PRODUCT AVAILABILITY**

IOTest CD69-PE Conjugated Antibody  
PN IM1943U – 2 mL Liquid – 20 µL / test\*.

PE is licensed under patent 4,520,110.

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

Outside the USA, contact your local Beckman Coulter representative.

[www.beckmancoulter.com](http://www.beckmancoulter.com)

**TRADEMARKS**

The Beckman Coulter logo and IOTest are trademarks of Beckman Coulter Inc.

Manufactured by:  
Immunotech, a Beckman Coulter Company  
130, avenue de Lattre de Tassigny, B.P. 177  
13276 Marseille Cedex 9, France

Copyright<sup>®</sup> Beckman Coulter, Inc. 2006  
All Rights Reserved

(\*) : 20 µL is the quantity of product sufficient to stain 5 x 10<sup>5</sup> cells in a standard immunofluorescence assay

