

### Analyte Specific Reagent.

Analytical and performance characteristics are not established.

#### SPECIFICITY

The CD69 molecule also designated as activation inducer molecule (AIM) is a phosphorylated disulfide linked 27/33 kDa homodimer composed of differentially glycosylated subunits. CD69 is a type II integral membrane protein with an extracellular C-type lectin domain (1).

It is the earliest inducible cell surface glycoprotein to appear upon « in vitro » activation of T cells, NK cells, and B cells. CD69 is involved in signal transduction during the initial steps of cell activation (2). CD69 is undetectable on the most circulating peripheral blood lymphocytes (3). Resting T cells do not express CD69 but its expression may be rapidly induced by triggering of their TCR/CD3 complex.

The majority of peripheral blood NK cells are negative for CD69, but they express AIM shortly after activation with PMA, IL-2,  $\alpha$  interferon, CD16 monoclonal antibody (4). CD69 is constitutively expressed in a sub-population of thymocytes and platelets (5). In NK cells and platelets CD69 acts as a triggering molecule. TP1.55.3 reacts with activated T lymphocytes but has weak or no effect on the induction of T-cell proliferation and IL-2 production, respectively (3). TP1.55.3 immunoprecipitates the 60 kDa homodimer and both 27 kDa and 33 kDa subunits from PBL activated (with PMA and anti-CD3 mAbs) under reduced and non-reduced conditions, respectively (6).

#### REAGENT

IOTest CD69-PC7  
Conjugated Antibody  
PN A80710 - 1 mL - Liquid

<b>Clone</b>	TP1.55.3
<b>Isotype</b>	IgG2b, Mouse
<b>Immunogen</b>	Activated Human PBL
<b>Hybridoma</b>	X63 x balb/c
<b>Source</b>	Ascites fluid or supernatant of in vitro cultured hybridoma cells.
<b>Purification</b>	Affinity chromatography
<b>Conjugation</b>	R Phycoerythrin-Cyanine 7 (PC7)
<b>Molar Ratio</b>	PC7 / Ig : 0.5 - 1.5
<b>Fluorescence</b>	Excites at 488 nm Emits at 770 nm

#### REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin. Concentration: See lot specific Certificate of Analysis at [www.beckmancoulter.com](http://www.beckmancoulter.com).

#### STATEMENTS OF WARNING

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 considered potentially infectious and disposed of with proper precautions.
3. Never pipet with 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.
7. Use good laboratory practices when handling this reagent.
8. Any change in the physical appearance of the reagents may indicate deterioration and the reagent should not be used.

#### STORAGE AND HANDLING CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

#### PRECAUTIONS

Due to the tandem structure of the fluorochrome, PC7 also emits light at 575 nm. This secondary emission peak varies from lot-to-lot of PC7. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a PC7-conjugate.

#### 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*, 10, 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.

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