

**Monoclonal Mouse  
Anti-Human CD69/RPE**  
Clone FN50  
**Code No. R 7173**

For research use only. Not for use in diagnostic procedures.

<b>Recommended use</b>	Monoclonal Mouse Anti-Human CD69 is recommended for use in flow cytometry for identification of cells expressing CD69.
<b>Introduction</b>	<p>CD69 is a phosphorylated disulphide-linked dimer composed of two chains of 27 kDa and 33 kDa, and also known as activation inducer molecule (AIM). CD69 is the earliest inducible surface antigen expressed on lymphocytes after T or B cell activation. CD69 is absent from resting lymphocytes (1). Other cells, including epidermal Langerhans cells, natural killer (NK) cells, eosinophils, neutrophils and platelets may also express CD69 (2). In vitro studies have demonstrated a transient expression of CD69 on activated T cells. After activation, surface expression can be detected within 2-4 hours, reaching a maximum after 18-24 hours followed by a gradual decrease (3). CD69 is thus detectable prior to other activation antigens like CD25 and CD71. CD69 is believed to be involved in signal transduction, since cross-linking with anti-CD69 induces activation (4).</p> <p>Anti-CD69 may be useful for T cell activation studies when used in combination with anti-CD4, anti-CD8, and anti-CD3.</p>
<b>Reagent provided</b>	<p>Purified monoclonal mouse antibody conjugated with R-phycoerythrin (RPE). The conjugate is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) and 15 mmol/L NaN<sub>3</sub>, pH 7.2. Each vial contains 100 tests (10 µL of conjugate for up to 10<sup>6</sup> leucocytes from normal human peripheral blood).</p> <p><u>Clone:</u> FN50 (5). <u>Isotype:</u> IgG1, kappa. <u>Conjugate concentration mg/L:</u> See label on vial.</p>
<b>Immunogen</b>	Activated B cells, isolated from peripheral blood (5).
<b>Specificity</b>	Anti-CD69, FN50, was included in the Fourth International Workshop and Conference on Human Leucocyte Differentiation Antigens and studies by a number of laboratories confirmed its reactivity with CD69 (6). Anti-CD69, FN50, was shown to label activated B and T cells (7). In lymph node and tonsil the antibody strongly labels intragerminal centre T cells located in the light (centrocyte-rich) zone. Additionally, the antibody labels many or most lymphocytes of the follicular mantle and perifollicular/interfollicular zones, and red pulp cord macrophages in spleen (5, 6).
<b>Precautions</b>	<ol style="list-style-type: none"><li>1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.</li><li>2. This product contains sodium azide (NaN<sub>3</sub>), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.</li><li>3. As with any product derived from biological sources, proper handling procedures should be used.</li></ol>
<b>Storage</b>	Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services.
<b>Staining procedure</b>	<ol style="list-style-type: none"><li>1. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube.</li><li>2. Add 10 µL of R 7173 and mix gently with a vortex mixer. The 10 µL is a guideline only; the optimal volume should be determined by the individual laboratory.</li><li>3. The recommended negative control is a non-reactive RPE-conjugated antibody of the same isotype.</li><li>4. Incubate in the dark at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15-30 minutes.</li><li>5. Add 100 µL of DakoCytomation Uti-Lyse™ (code Nos. S 3325 or S 3350) Reagent A to each sample and mix gently with a vortex mixer. Incubate for 10 minutes at room temperature in the dark.</li><li>6. Add 1 mL of DakoCytomation Uti-Lyse™ Reagent B to each sample and mix gently with a vortex mixer. Incubate for 10 minutes at room temperature in the dark. If another lysing reagent is used in steps 5 and 6, please follow the recommendations for that reagent.</li><li>7. Centrifuge at 300 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.</li><li>8. Add 2 mL 0.01 mol/L PBS containing 2% bovine serum albumin and resuspend the cells by using a vortex mixer.</li><li>9. Repeat step 7.</li></ol>

10. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS. The PBS should contain 1% paraformaldehyde (fixative) if samples are not analysed the same day.
11. Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. It is recommended to include a suitable positive and negative control sample with each run for reagent and preparation control. Note that fluorochrome conjugates are light sensitive, and samples should be protected from light during the staining procedure and until the analysis.

## References

1. Sánchez-Madrid F. AA4. Overview of CD69. In: Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, et al., editors. Leukocyte typing V. White cell differentiation antigens. Proceedings of the 5th International Workshop and Conference; 1993 Nov 3-7; Boston, USA. Oxford, New York, Tokyo: Oxford University Press; 1995. Volume 1, p. 1123-4.
2. Poggi A. CD Guide. CD69. In: Mason D, André P, Bensussan A, Buckley C, Civin C, Clark E, et al., editors. Leucocyte typing VII. White cell differentiation antigens. Proceedings of the 7th International Workshop and Conference; 2000 Jun 19-23; Harrogate, United Kingdom. New York: Oxford University Press Inc.; 2002. p. 821-2.
3. Hara T, Jung LK, Bjorndahl JM, Fu SM. Human T cell activation. III. Rapid induction of a phosphorylated 28 kD/32 kD disulfide-linked early activation antigen (EA 1) by 12-o-tetradecanoyl phorbol-13-acetate, mitogens, and antigens. J Exp Med 1986;164:1988-2005.
4. Testi R, Phillips JH, Lanier LL. T cell activation via Leu-23 (CD69). J Immunol 1989; 143:1123-8.
5. Beiske K, Aas-Eng DA, Smeland EB, Sundan A, Marton PF, Funderud S. A4.2. Immunohistological and functional characterization of the mAb A91 (FN 50)-reactive activation antigen (CD69) expressed on subsets of normal and neoplastic lymphoid cells. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 436-9.
6. Schwarting R, Niedobitek G, Stein H. A4. Cluster report: CD69. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 428-32.
7. Cebrián M, Sánchez-Mateos P, Redondo JM, Ursa A, De Landázuri MO, Sánchez-Madrid F. A4.4. 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. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 441-4.

## Explanation of symbols

 REF	Catalogue number		Keep away from sunlight (consult storage section)		Manufacturer
	Consult instructions for use		Batch code		
	Temperature limitation		Use by		