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Product	CD63 FITC
Cat. N°	CD63FITCTEA3/18
Reagent provided	100test (20µl/test)
Lote	
Isotype	IgG1
Clone	TEA3/18 (3° Internacional Workshops on HLDA, Code P-47)
Fluorochrome	Fluorescein isothiocyanate (Molecular Probes)
Decription	Monoclonal Mouse Anti-Human CD63 FITC, is recommended for use in flow cytometry for identification of activated platelets. The conjugate is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) (Lote: 113K1364 / SIGMA) and 0,09% NaN ₃ , pH 7.2.
Storage	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.
Expire date	2007-11
Specificity	CD63 is a 53 kD, type III lysosomal glycoprotein, expressed on activated platelets, monocytes and macrophages. This molecule is also referred highly to as LIMP, gp55, melanoma-associated antigen ME491, Pltgp40, LAMP-3 and is a member of the tetraspan transmembrane 4 superfamily (TM4SF). It is also widely expressed on surface and in the cytoplasm of various hematopoietic (monocytes, macrophages) and non-hematopoietic cells (endothelium, fibroblasts, osteoclasts, smooth muscle). Its cellular function has not been fully elucidated. Reports suggest this as a useful molecule to study platelet activation.
Reactivity	The monoclonal antibody is directed against the CD63- antigen, which is expressed in platelet lysosomes that is translocated to the platelet surface upon activation with strong agonists. The antigen is also present in most peripheral blood cells (not in erythrocytes) and in many tissues; both surface and cytoplasmatic locations are reported.
Cross reactivity	Not Known.
Aplication	It is recommended for use in flow cytometry This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 20 µl/5 µl platelets.
Precautions	<ol style="list-style-type: none"> 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. 2. This product contains sodium azide (NaN₃), 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. 3. As with any product derived from biological sources, proper handling procedures should be used.



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Staining procedure

Preparation

- A. Procedure for preparation of ACTIVATED PLATELETS.
1. Centrifuge tube of freshly drawn EDTA blood (600rpm or 75xg, 20')
 2. Remove platelets (top layer) and wash twice over in PBS and resuspend in PBS.
 3. Add thrombin to cell suspension (1-0,2 U/ml) or PMA (25µg/ml).
 4. Add 20 µL of CD63 FITC and mix gently with a vortex mixer. The 20 µL is a guideline only; the optimal volume should be determined by the individual laboratory.
 5. The recommended negative control is a non-reactive FITC-conjugated antibody of the same isotype. (Code No. ISOCONFITCIGG1).
 6. Incubate in the dark at room temperature at room temperature (20-25 °C) for 15 minutes.
 7. Incubate in the dark at 4 °C for 10 minutes.
 8. Analyse on a flow cytometer.

- B. Procedure for preparation of RESTING PLATELETS:
1. Centrifuge tube of freshly drawn EDTA blood (600rpm or 75xg, 20')
 2. Remove platelets (top layer) and wash twice over in PBS and resuspend in PBS.
 3. Add 20 µL of CD63 FITC and mix gently with a vortex mixer. The 20 µL is a guideline only; the optimal volume should be determined by the individual laboratory.
 4. The recommended negative control is a non-reactive FITC-conjugated antibody of the same isotype. (Code No. ISOCONFITCIGG1).
 5. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
 6. Analyse on a flow cytometer.

FOR MORE INFORMATION, please visit our website: www.citometriadeflujo.info

References

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Luis Escribano, Beatriz Díaz-Agustín, Rosa Núñez, Aranzazu Prados, Ramón Rodríguez, Alberto Orfao. *International Archives of Allergy and Immunology* 2002;127:127-132 (DOI: 10.1159/000048183).
2. Azorsa, D.O., J.A. Hyman, and J.E.K. Hildreth. 1991. CD63/Pltgp40: A platelet activation antigen identical to the stage-specific, melanoma-associated antigen ME491. *Blood* 78: 280.
3. Hildreth, J.E.K., D. Derr, and D.O. Azorsa. 1991. Characterization of a novel self-associating Mr 40,000 platelet glycoprotein. *Blood* 77: 121
4. Schlossman, S., L. Bloumsell, W. Gilks, et al., eds. 1995. Leucocyte Typing V: White Cell Differentiation Antigens. Oxford University Press, New York.
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