

**Monoclonal Mouse
Anti-Human
CD54, ICAM-1/FITC**
Clone 6.5B5
Code No. F 7143

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








Recommended use	Monoclonal Mouse Anti-Human CD54, ICAM-1/FITC, is recommended for use in flow cytometry for identification of cells expressing CD54.
Introduction	The CD54 antigen is a single chain glycoprotein with a molecular mass of 90 kDa (1). It is the human intercellular adhesion molecule-1 (ICAM-1) belonging to the immunoglobulin supergene family. The ligand for ICAM-1 is LFA-1 (leucocyte function associated-1 protein) (2). ICAM-1 is widely expressed on many cell types, although the degree of expression by unstimulated resting cells varies. As a cellular activation antigen the expression of ICAM-1 is up-regulated upon cell activation, being induced particularly in response to cytokines such as interleukin-1, tumour necrosis factor and interferon- γ (2-4). ICAM-1 might be a receptor for rhinovirus (5). In rejecting kidneys the antibody stains all infiltrating cells strongly as well as glomerulus epithelium, endothelium on capillaries, large vessels and mesangium (6).
Reagent provided	Purified monoclonal mouse antibody conjugated with fluorescein isothiocyanate isomer 1 (FITC). The conjugate is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) and 15 mmol/L NaN_3 , pH 7.2. Each vial contains 100 tests (10 μL of conjugate for up to 10^6 leucocytes from normal human peripheral blood). <u>Clone:</u> 6.5B5 (3). <u>Isotype:</u> IgG1, kappa. <u>Conjugate concentration mg/L:</u> See label on vial.
Immunogen	TNF-activated human umbilical vein endothelial cells.
Specificity	Anti-CD54, 6.5B5, was included in the Sixth International Workshop and Conference on Human Leucocyte Differentiation Antigens, and studies by a number of laboratories confirmed its reactivity with the CD54 antigen (7). Anti-CD54, 6.5B5, reacts with domain 1 (nearest the N-terminal of the molecule) of ICAM-1. It is mainly expressed on leucocytes, on epithelial and on endothelial cells (3, 4, 8-10).
Precautions	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 (NaN_3), 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.
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.
Staining procedure	<ol style="list-style-type: none">1. Transfer 100 μL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube.2. Add 10 μL of F 7143 and mix gently with a vortex mixer. The 10 μL is a guideline only; the optimal volume should be determined by the individual laboratory.3. The recommended negative control is a non-reactive FITC-conjugated antibody of the same isotype.4. Incubate in the dark at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15-30 minutes.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.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.7. Centrifuge at 300 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 μL of fluid.8. Add 2 mL 0.01 mol/L PBS containing 2% bovine serum albumin and resuspend the cells by using a vortex mixer.9. Repeat step 7.10. 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. Springer TA. Adhesion receptors of the immune system. *Nature* 1990;346:425-34.
2. Berendt AR, McDowall A, Craig AG, Bates PA, Sternberg MJE, Marsh K, et al. The binding site on ICAM-1 for *Plasmodium falciparum*-infected erythrocytes overlaps, but is distinct from, the LFA-1-binding site. *Cell* 1992;68:71-81.
3. Wellicome SM, Thornhill MH, Pitzalis C, Thomas DS, Lanchbury JSS, Panayi GS, et al. A monoclonal antibody that detects a novel antigen on endothelial cells that is induced by tumor necrosis factor, IL-1 or lipopolysaccharide. *J Immunol* 1990;144:2558-65.
4. Thornhill MH, Kyan-Aung U, Haskard DO. IL-4 increases human endothelial cell adhesiveness for T cells but not for neutrophils. *J Immunol* 1990;144:3060-5.
5. Staunton DE, Merluzzi VJ, Rothlein R, Barton R, Marlin SD, Springer TA. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* 1989;56:849-53.
6. Waugh JA, Bishop GA, Aversa GG, Sebley RK, Hall BM. A 8.8 staining of normal or rejecting kidney using the activation panel. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. *Leucocyte 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. 485-6.
7. Imai K, Tsujisaki M, Hareyama M, Hinoda Y. AS20. CD54 Workshop panel report. In: Kishimoto T, Kikutani H, von dem Borne AEG, Goyert SM, Mason DY, Miyasaka M, et al., editors. *Leucocyte typing VI. White cell differentiation antigens. Proceedings of the 6th International Workshop and Conference; 1996 Nov 10-14; Kobe, Japan.* New York, London: Garland Publishing Inc.; 1997. p. 409-11.
8. Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am J Pathol* 1992;140:665-73.
9. Norris P, Poston RN, Thomas DS, Thornhill M, Hawk J, Haskard DO. The expression of endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in experimental cutaneous inflammation: a comparison of ultraviolet B erythema and delayed hypersensitivity. *J Invest Dermatol* 1991;96:763-70.
10. Garioch JJ, Mackie RM, Campbell I, Forsyth A. Keratinocyte expression of intercellular adhesion molecule 1 (ICAM-1) correlated with infiltration of lymphocyte function associated antigen 1 (LFA-1) positive cells in evolving allergic contact dermatitis reactions. *Histopathology* 1991;19:351-4.

Explanation of symbols

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