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ProductInformation

MONOCLONAL ANTI-HUMAN CD54 (ICAM-1), CLONE 8.4A6, FITC CONJUGATE

Product Number **F 0549**

Product Description

Monoclonal Anti-Human CD54 (ICAM-1) (mouse IgG1 isotype) is derived from the 8.4A6 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with TNF-α activated human endothelial cells.[1] The immunoglobulin fraction of antibody to CD54 is purified (protein A) from ascites fluid and then conjugated to fluorescein isothiocynate (FITC) isomer I. The conjugate is purified by gel filtration and contains no detectable free FITC.

Monoclonal Anti-Human CD54 (ICAM-1) – FITC Conjugate reacts specifically with CD54 (ICAM-1) expressed on the surface of activated endothelial cells, lymphocytes, monocytes and granulocytes. The epitope recognized by the antibody is localized in domain D2 of the CD54 (ICAM-1) molecule.[2] The product may be used in flow cytometry.

Human CD54, also known as intercellular adhesion molecule-1 (ICAM-1), is a 85-110 kDa single-chain type 1 integral membrane glycoprotein with an extra-cellular domain of five immunoglobulin superfamily repeats, a transmembrane region and a cytoplasmic domain. It shares considerable amino acid sequence homology with CD50 (ICAM-3) and with CD102 (ICAM-2). CD54 (ICAM-1) is expressed by activated endothelial cells. It is detected on cells of many other lineages (e.g. epithelial cells, fibroblasts, chondrocytes, B lymphocytes, T lymphocytes (low), monocytes, macrophages, dendritic cells, and neutrophils, with lower levels that increase in inflammation.[1, 2, 4] Also, CD54 (ICAM-1) is expressed in some carcinoma and melanoma cells.[2] Soluble CD54 (ICAM-1) is detectable in the plasma and is elevated in patients with various inflammatory syndromes.[3] CD54 (ICAM-1) mediates myeloid cells adhesion to activated vascular endothe-lium at the vessel wall and to other leucocytes.[1, 2] CD54 (ICAM-1) mediates T cell interactions with Antigen Presenting Cells or target cells and other T-T or T-B cell interactions CD54 (ICAM-1) is the ligand for the integrin LFA-1 (CD11a/CD18).[4] In addition, it has binding sites for Rhinovirus,[5] Mac-1 (CD11b/CD18) [6] and *Plasmodium falciparum*-infected erythrocytes.[7] It seems to serve as an adhesion receptor for *Plasmodium falciparum*, thereby enabling binding of infected red blood cells to the vascular endothelia in selected organs. This causes the typical sequestration of severe malaria. *In vitro* upregulation of CD54 (ICAM-1) expression of cultured endothelial cells occurs upon activation of various inflammatory mediators, such as IL-1β, TNF-α, or IFN-γ. It is induced by phorbol esters, retinoic acid and lipopolysaccharides. Peak levels of CD54 (ICAM-1) usually occur within 10-24 hours.[1] The 8.4A6 producing hybridoma was developed by D.O. Haskard and coworkers[1] at the Rheumatology Unit, the Division of Medicine, Guy's Hospital, London

Reagents

The product is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

The F/P molar ratio of the product is 3-8.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C. **Protect from prolonged exposure to light**. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

When assayed by flow cytometric analysis (FACScan), using 10µl of the antibody to stain 2 x 10^5 cells (rhTNF-α activated human umbilical cord vein endothelial cells, HUVEC), a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. Also, the percent population positive is at the maximum percentage positive using saturating monoclonal antibody levels.

A minimum working dilution of 1:10 is determined by direct immunofluorescence using acetone-fixed frozen human tonsil sections.

Notes:

1. In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.
2. It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary antibody. The ideal negative control reagent is an FITC conjugated mouse monoclonal antibody or myeloma protein. It should be isotype-matched, F/P molar ratio-matched, not specific for the tested preparation, and of the same concentration as the tested antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of preparation under study and the sensitivity of the instrument used. For fluorescent analysis of preparation expressing Fc receptors, the use of isotype-matched negative controls is mandatory.

References

1. Wellicome, S.M., et al., J.Immunol., **144**, 2558 (1990).
2. Leukocyte Typing V, Schlossman, S.F., et al., (eds.), pp. 1509,1544, 1548-1550, 1552, 1554-1557, 1579-1581, Oxford University Press, Oxford, (S100) (1995).
3. Mason, J.C., et al., Arthritis Rheum., **36**, 519 (1993).
4. Springer, T.A., et al., Nature, **346**, 425 (1990).
5. Staunton, D.E., et al., Cell, **61**, 243 (1990).
6. Diamond, M.S., et al., Cell, **65**, 961 (1991).
7. Berendt, ,A.R., et al., Cell, **68**, 71 (1992).

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