

## Product Information

### MONOCLONAL ANTI-HUMAN ICAM-1 (CD54),

#### Clone 8.4A6

Purified Mouse Immunoglobulin

Product Number **C2969**

#### Product Description

Monoclonal Anti-Human ICAM-1 (CD54) (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 a TNF- $\alpha$  activated human endothelial cells.<sup>1</sup> The 8.4A6 producing hybridoma was developed by D.O. Haskard and coworkers<sup>1</sup> at the Rheumatology Unit, the Division of Medicine, Guy's Hospital, London.

The isotype is determined using Sigma ImmunoType Kit<sup>™</sup> (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The antibody is purified from ascites fluid using protein A.

Monoclonal Anti-Human ICAM-1 (CD54) reacts specifically with ICAM-1 (CD54) 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 ICAM-1 (CD54) molecule.<sup>2</sup> The antibody may be used in flow cytometry, ELISA,<sup>3</sup> immunocytochemistry,<sup>2</sup> and immunohistochemistry (frozen tissues). It inhibits leukocytes adhesion to activated endothelial cells,<sup>2</sup> but not adhesion of *Plasmodium falciparum*-infected erythrocytes.<sup>4</sup> The antibody stimulates homotypic aggregation of SKW3 cells.<sup>2</sup> It is also useful for studying soluble CD54 (ICAM-1) using capture ELISA.<sup>3</sup>

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 extracellular 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).<sup>5,6</sup> ICAM-1 (CD54) 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.<sup>1,2,5</sup> Also, ICAM-1 (CD54) is detected in some carcinoma and melanoma cells.<sup>2</sup> Soluble ICAM-1 (CD54) is detectable in the plasma and is elevated in patients with various inflammatory syndromes.<sup>3</sup> ICAM-1 (CD54) mediates myeloid cells adhesion to activated vascular endothelium at the vessel wall and to other leucocytes.<sup>1,2</sup> ICAM-1 (CD54) mediates T cell interactions with antigen presenting cells or target cells and also other T-T or T-B cell interactions. It seems to serve as an adhesion receptor for *Plasmodium falciparum*, thereby enabling binding of infected RBC to the vascular endothelia in selected organs, causing the typical sequestration of severe malaria. ICAM-1 (CD54) is the ligand for the integrin LFA-1 (CD11a/CD18).<sup>5</sup> In addition, ICAM-1 has binding sites for Rhinovirus,<sup>6</sup> Mac-1(CD11b/CD18)<sup>7</sup> and *Plasmodium falciparum*-infected erythrocytes.<sup>4</sup> *In vitro* upregulation of CD54 (ICAM-1) expression of cultured endothelial cells occurs upon activation by various inflammatory mediators, such as IL-1 $\beta$ , TNF- $\alpha$  or IFN- $\gamma$ . It is induced by phorbol esters, retinoic acid, and lipopolysaccharides. Peak levels of ICAM-1 usually occur within 10-24 hours.<sup>1</sup>

#### Reagents

The product is supplied sterile-filtered, in 0.01 M phosphate buffered saline, pH 7.4.

Antibody concentration is approximately 2 mg/ml as determined by E<sub>280</sub>.

#### Storage/Stability

For continuous use, store sterile at 2-8°C. For extended storage freeze in sterile working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

### Product Profile

A minimum working dilution of 10-20 µg/ml is determined by indirect immunofluorescent staining of acetone-fixed, frozen human tonsil sections.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

### References

1. Wellicome, S.M., et al., J.Immunol., **144**, 2558 (1990).
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3. Mason, J.C., et al., Arthritis Rheum., **36**, 519 (1993).
4. Berendt, A.R., et al., Cell, **68**, 71 (1992).
5. Springer, T.A., et al., Nature, **346**, 425 (1990).
6. Staunton, D.E., et al., Cell, **61**, 243 (1990).
7. Diamond, M.S., et al., Cell, **65**, 961 (1991).

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