

## Reagents Provided

**Alexa Fluor<sup>®</sup> 700-conjugated rat monoclonal anti-mouse VCAM-1/CD106:** Supplied as 25 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

**Clone #:** 112734

**Isotype:** rat IgG<sub>2a</sub>

## Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

## Storage

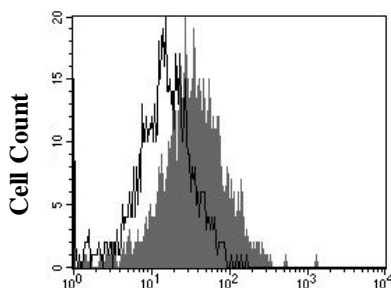
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing VCAM-1/CD106 within a population and qualitatively determine the density of VCAM-1/CD106 on cell surfaces by flow cytometry.

## Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified NS0-derived recombinant mouse VCAM-1/CD106 (aa 25-698; Accession # P29533). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor<sup>®</sup> 700 fluorochrome. Cell surface expression of VCAM-1/CD106 is determined by flow cytometry using 675-700 nm wavelength excitation range and monitoring emitted fluorescence with a detector optimized to collect peak emissions at around 723 nm.



VCAM-1/CD106-Alexa Fluor<sup>®</sup> 700

Mouse bEnd.3 cells were stained with Alexa Fluor<sup>®</sup> 700-conjugated anti-mouse VCAM-1/CD106 (Catalog # FAB6432N; filled histogram) or Alexa Fluor<sup>®</sup> 700-conjugated isotype control (Catalog # IC006N; open histogram).

## Legal

This product is provided under an agreement between Life Technologies Corporation and R&D Systems, Inc. and the manufacture, use, sale or import of this product is subject to one or more US patents and corresponding non-US equivalents, owned by Life Technologies Corporation and its affiliates. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components (1) in manufacturing; (2) to provide a service, information, or data to an unaffiliated third party for payment; (3) for therapeutic, diagnostic or prophylactic purposes; (4) to resell, sell, or otherwise transfer this product or its components to any third party, or for any other commercial purpose. Life Technologies Corporation will not assert a claim against the buyer of the infringement of the above patents based on the manufacture, use or sale of a commercial product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, Cell Analysis Business Unit, Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Background Information

VCAM-1 (CD106), a member of the immunoglobulin superfamily, is a cell surface protein expressed by activated endothelial cells and certain leukocytes (such as macrophages). VCAM-1 expression is induced by IL-1β, IL-4, TNF-α, and IFN-γ. VCAM-1 binds to leukocyte integrins VLA4 and α4β7. The human and mouse VCAM-1 proteins share approximately 76% amino acid similarity. During the inflammatory adhesion mechanism, activated integrins halt rolling leukocytes and attach them firmly to the vascular endothelium. They do this by binding to their ligands, for example VCAM-1, on endothelium. The VCAM-1:VLA4/Integrin α4β7 interaction is also thought to be involved in the extravasation of white blood cells through the blood vessel wall to sites of inflammation. ELISA techniques have shown that detectable levels of soluble VCAM-1 are present in the biological fluids of apparently normal individuals. Furthermore, a number of studies have reported that levels of VCAM-1 may be elevated or lowered in subjects with a variety of pathological conditions.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse bEnd.3 cells.

- Cells may be Fc-blocked with 1 µg of mouse IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 5 µL of conjugated antibody was added to up to 1 x 10<sup>6</sup> cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Mouse Lyse Buffer (Catalog # FC003).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor<sup>®</sup> 700-labeled rat IgG<sub>2a</sub> antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

**Warning:** Contains sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.