

# **MOLECULAR PROBES®**

# PRODUCT INSERT

## MONOCLONAL ANTIBODY TO THE MOUSE CD106 (VCAM-1) ANTIGEN

| Product   | Form | Volume | Antibody* | Excitation    | Peak Emission | Matching Isotype Controls |      |
|-----------|------|--------|-----------|---------------|---------------|---------------------------|------|
|           |      |        |           | ( <b>nm</b> ) | ( <b>nm</b> ) |                           |      |
| RMCD10601 | FITC | 1.0 ml | 500 μg    | 488           | 525           | Rat IgG1 FITC             | R101 |
| RMCD10604 | R-PE | 1.0 ml | 100 µg    | 488           | 575           | Rat IgG1 R-PE             | R104 |

## PRODUCT DESCRIPTION

Rat monoclonal antibody to the mouse CD106 (VCAM-1) antigen

Clone: M/K-2

Isotype: Rat IgG<sub>1</sub>κ

Lot No.: See label Expiration: See label

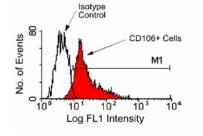
**Buffer:** Phosphate buffered saline (PBS)

**Preservatives:** 0.1% *sodium azide*. Sodium azide is an extremely toxic and dangerous compound particularly when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

Stabilizer: Sucrose.

## PRODUCT CHARACTERIZATION

Antigen Specificity: VCAM-1 is an adhesion molecule and a major mediator of the inflammatory response. It is expressed on activated microvascular endothelial cells in response to signals arising from immune responses in infection, graft rejection, tumor recognition and killing. The complementary binding ligand for VCAM-1 is VLA-4/CD49d. In addition to VCAM-1, VLA-4 also recognizes the extracellular matrix molecule fibronectin. This pairing of VCAM-1 and VLA-4 is able to provide a second signal (i.e., non-antigen specific) for T cell stimulation, such as that seen in transplantation. The monoclonal antibody MK-2 has been used in transplant studies to suppress cardiac rejection and induce long-term cardiac graft survival. In addition to inflammatory responses, VCAM-1 has a significant role in hemopoiesis through its ability to retain lymphocyte and myeloid precursors on stromal cells in the marrow and lymphoid organs. CD106/VCAM-1 exists as an integral membrane protein. The M/K-2 monoclonal antibody immunoprecipitates a peptide that gives a single band on SDS-PAGE gels with an apparent Mr of ~100 kDa under reducing conditions and 92 kDa under nonreducing conditions.<sup>1-5</sup>



BALB/c bone marrow cells were stained with rat anti-mouse CD106/VCAM-1-FITC, following which large cells were gated and analyzed by flow cytometry.

### **Research Applications:**

- Identification and enumeration of CD106 cells by flow cytometry<sup>1-5</sup>
- Identification of VCAM-1<sup>+</sup> cells by immunofluorescence and immunohistochemical staining <sup>1-5</sup>
- Blockage of lymphocyte adhesion in Whitlock-Witte long-term bone marrow cultures <sup>1,2</sup>
- Inhibition of lymphopoiesis in Whitlock-Witte cultures <sup>1, 2</sup>
- Immunoprecipitation<sup>1</sup>

**Note:** Flow cytometric data shown may not necessarily have been generated using the enclosed lot of reagent. For this reason, and due to differences in flow cytometers and cytometer settings, results may vary from those illustrated above. It is suggested that investigators titrate reagents to determine optimal conditions for use in their systems.

#### **STORAGE & HANDLING**

Store reagents at  $2-8^{\circ}$ C. Light exposure should be avoided for fluorochrome-conjugated reagents. Use dim light during handling, incubation with cells and prior to analysis. It is recommended that cells be analyzed within 18 hours of staining. If the reagent is being diluted, it is recommended that only the quantity to be used within one week be diluted.

## PRODUCT QUALITY CONTROL

To ensure lot-to-lot consistency, each batch of monoclonal antibody is tested by flow cytometry to conform to characteristics of a standard reference reagent. From this testing it is recommended that between 0.1 and 0.2  $\mu$ g of antibody be used per 1 x 10<sup>6</sup> cells in a 100  $\mu$ l staining volume. Because conditions may vary, it is recommended that each investigator determine the optimal amount of antibody to be used for each application.

\* The amount of antibody is determined by measuring the optical density using a spectrophotometer. The antibody titer is verified by immunofluorescent staining and flow cytometric analysis.

## **REFERENCES:**

1. Miyake, K., I.L. Weissman, J.S. Greenberger and P. W. Kincade. 1991. J. Exp. Med. 173:599.

(Rev 02/09) DCC-09-0140

- 2. Miyake, K., et al.. 1991. J. Cell. Biol. 114(3):557.
- 3. Schlegel, P.G., et al. 1995. J. Immunol. 155:3856.
- 4. Bergese, S., et al. 1995. Am J. Phathol. 146:989.
- 5. Orosz, C.G., et al. 1993. Transplantation 56:453.

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#### PI L13038

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