

## Product Information

### **MONOCLONAL ANTI-HUMAN CD11C CLONE 3.9 Biotin Conjugate Purified Mouse Immunoglobulin**

Product No. **B 0656**

#### **Product Description**

Monoclonal Anti-Human CD11c (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes of BALB/c mice immunized with rheumatoid synovial fluid cells followed by fibronectin purified human monocytes.<sup>1,2</sup> The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of  $\epsilon$ -amino caproyl biotin to purified CD11c monoclonal antibody.

Monoclonal Anti-Human CD11c antibody recognizes the 150 kD,  $\alpha$ -chain of the CD11c/CD18 complex, an  $\alpha/\beta$  heterodimeric glycoprotein which belongs to the  $\beta_2$  integrin family.<sup>1-3</sup> It is also known as p150,95. Human CD11c is constitutively present on monocytes, macrophages, dendritic reticular cells, NK cells and certain cytotoxic T cells.<sup>2-7</sup> Low levels of CD11c are expressed on polymorphonuclear cells. It is strongly expressed on hairy cell leukemia cells and carried on B cell prolymphocytic leukemia cells. Expression on a B cell subset is weak. A large proportion of CD11c/CD18 is stored in intracellular compartments and translocated to the cell surfaces upon stimulation by inflammatory mediators, resulting in enhanced adhesiveness of the cells to vascular endothelial cells. CD11c/CD18 is a lipopolysaccharide receptor and mediates the attachment of unopsonized bacteria and fungi to leukocytes. Monoclonal Anti-CD11c stains macrophages in acetone-fixed, frozen sections of human tissues (e.g., lung, brain, colon, skin, spleen, thymus, tonsil). The epitope recognized by the antibody is formalin sensitive.

The antibody reduces adhesion of TNF-stimulated polymorphonuclear cells to surfaces coated with fibrinogen.<sup>8</sup>

#### **Reagents**

The conjugate is provided as purified antibody (50  $\mu$ g/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

#### **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 hazardous and safe handling practices.

#### **Uses**

Biotin Conjugated Monoclonal Anti-Human CD11c may be used for:

1. Characterization of leukemias and lymphomas.
2. Detection and monitoring of leukocyte adhesion deficiencies.
3. Studies of cell adhesion and effector-target interactions.

#### **Performance**

When assayed by flow cytometric analysis, using 10  $\mu$ l of the antibody to stain  $1 \times 10^6$  cells, a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

In order to obtain best results in different techniques and preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

## Storage

Store at 2-8 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

## Procedure for Indirect Immunofluorescent Staining using Biotinylated Primary Antibodies

### Reagents and Materials Needed but Not Supplied

- Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant **or**
  - Human cell suspension (e.g., peripheral blood mononuclear cells isolated on HISTOPAQUE<sup>®</sup> Product Code 1077-1).
- Diluent: 0.01 M Phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0-.1% NaN<sub>3</sub>.
- Fluorochrome (FITC, PE, or Quantum Red<sup>™</sup>) conjugated avidin derivative diluted to recommended working dilution in diluent. Appropriate products for use are ExtrAvidin<sup>®</sup>-FITC (Product No. E2762), Streptavidin-FITC (Product No. S3402), Streptavidin-PE (Product No. S3762), or Streptavidin-Quantum Red<sup>™</sup> (Product No. S2899).
- 12 x 75 mm test tubes.
- Adjustable micropipet.
- Centrifuge.
- Counting chamber.
- 0.2% Trypan blue (Product No. T0776) in 0.01 M phosphate buffered saline, pH 7.4.
- 2% paraformaldehyde in PBS.
- Whole blood lysing solution.
- Flow cytometer.

### Procedure

- Use 100 µl of whole blood **or**
  - Adjust cell suspension to  $1 \times 10^7$  cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue). For each sample, add 100 µl or  $1 \times 10^6$  cells per tube.
- Add 10 µl of biotinylated monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 - 22°C) for 30 minutes.
- After 30 minutes, add 2 ml of diluent to all tubes.
- Pellet cells by centrifugation at 500 x g for 10 minutes.
- Remove supernatant by careful aspiration.
- Resuspend cells in 2 ml diluent.
- Repeat washing procedure (steps 4-6) twice.
- After the last wash, resuspend the cells in 100 µl of the fluorochrome conjugated avidin derivative at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 - 22°C) for 30 minutes. Protect from light at this and all subsequent steps.
- If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10.
  - If a mononuclear cell suspension is used, proceed to Step 10.
- Centrifuge and wash as in steps 4 - 6 twice.
- After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored before analyzing) and analyze in a flow cytometer according to manufacturer's instructions.

## References

1. Leucocyte Typing III, Oxford University Press, p. 795 (1987).
2. Hogg, N., et al., Eur. J. Immunol., **16**, 240 (1986).
3. Malhotra, V., et al., Eur. J. Immunol. **16**, 1117 (1986).
4. Sanchez-Madrid, F., et al., J. Exp. Med., **158**, 1785 (1983).
5. Lanier, L., et al., Eur. J. Immunol., **15**, 713 (1985).
6. Myones, B., et al., J. Clin. Invest., **82**, 640 (1988).
7. Stacker, S., and Springer, T., J. Immunol., **146**, 648 (1991).
8. Loike, J., et al., PNAS, USA., **88**, 1044 (1991).

9/98

Sigma brand products are sold through Sigma-Aldrich, Inc.  
Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.  
Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.  
Please see reverse side of the invoice or packing slip.