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ProductInformation

MONOCLONAL Anti-Human CD62L
CLONE FMC46
Purified Mouse Immunoglobulin

Product No. **C0590**

Product Description

Monoclonal Anti-Human CD62L (L-Selectin) (mouse IgG2b isotype) is derived from the FMC46 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Human PHA-activated peripheral blood mononuclear cells were used as the immunogen. 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 human CD62L (L-Selectin, LAM-1, Leu8, TQ1, LECAM-1, LECCAM-1, DREG, lymph node homing receptor, MEL-14 Ag) is a 74-95 kD glycoprotein member of the selectin family of adhesion receptors. L-Selectin is comprised of an amino-terminal C-type lectin binding domain, an epidermal growth factor-like domain, two short consensus repeat (SCR) sequences homologous to those found in complement binding proteins, a short spacer region, a transmembrane region and a short cytoplasmic region. Human CD62L (L-Selectin) is constitutively expressed on all classes of leukocytes including lymphocytes (except a substantial population of memory T cells), monocytes and polymorphonuclear cells. It is expressed on bone marrow myeloid progenitor cells, erythroid precursor cells and some thymocytes.[1,2,3] It is preferentially localized to the tips of lymphocytes and polymorphonuclear cells microvilli. Bioactive soluble L-Selectin derived from lymphocytes and neutrophils is present in normal and pathological biological fluids. Human CD62L (L-Selectin) binds to Sialyl Lewis^x, Sialyl Lewis^a, sulfatide, heparin, heparin sulfate, proteoglycans, fucoidan, dextran sulfate, yeast polyphosphomannan monoester core polysaccharide (PPME), carbohydrates presented on CD34 scaffold and to leukocyte P-Selectin glycoprotein Ligand-1 (PSGL-1). It is anchored to the cell cytoskeleton through interaction between its cytoplasmic tail and a-actinin. CD62L (L-Selectin) is shed by proteolytic cleavage from the surface of *in vitro* activated lymphocytes and neutrophils and *in vivo* from neutrophils during inflammation. Human CD62L (L-Selectin) mediates the transient calcium dependent binding of lymphocytes to specialized high endothelial cells in post capillary venules (HEV) of lymph nodes which are involved in lymphocyte homing and recirculation. CD62L (L-Selectin) also mediates leukocyte >rolling= on activated endothelial cells at sites of tissue injury and inflammation. In frozen tissue sections, Monoclonal Anti-Human CD62L (L-Selectin) stains follicles and lymphocytes around blood vessels in spleen, medulla and subcapsular areas in thymus and mantle zone, and to a lesser extent, germinal centers in lymph nodes and tonsil. The epitope recognized by the antibody is localized to the lectin domain of L-Selectin. It is sensitive to routine formalin-fixation and paraffin-embedding. The antibody partially inhibits >rolling= interactions between L-Selectin transfected L1-2 cells and a purified peripheral node addressin.[4] It significantly reduces binding of FITC conjugated PPME to these cells as well as to blood lymphocytes, monocytes and neutrophils.[4] The antibody producing hybridoma was developed by H. Zola[1] and Coworkers at the department of Clinical Immunology, Flinders Medical Center, Adelaide, Australia.

Reagents

The product is provided as purified antibody in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15mM 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.

Specificity
Monoclonal Anti-Human CD62L (L-Selectin) recognizes the human CD62L antigen expressed on neutrophils, monocytes, NK cells, B and certain T lymphocytes.

Uses
Monoclonal Anti-Human CD62L (L-Selectin) is a homogenous population of antibody molecules which may be used for:

1. Detection and enumeration of CD62L cells in blood and tissues in health and disease.
2. Studies of CD62L (L-Selectin) function in cell-cell interactions.

References

1. Pilarski, L.M., et al., J. Immunol., **147**, 136 (1991).
2. Tedder, T.F., et al., J. Immunol., **144**, 532 (1995).
3. Barclay, A.N., et al., in The Leukocyte Antigen Facts Book, Academic Press, London, pp. 348 (1993).
4. Leucocyte Typing V, Schlossman, S.F., et al., (eds). Oxford University Press, Oxford, pp. 1499, 1503, 1509, 1515, 1517 (S061) (1995).

Procedure for Indirect Immunofluorescent Staining
Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant **or**
   b. Human cell suspension (e.g. peripheral blood mononuclear cells isolated on HISTOPAQUE® (Product Code 1077-1)).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Product No. F2883 FITC-Sheep Anti-Mouse IgG, F(ab?)$_2$ fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. M5534).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Product No. T 0776) in 0.01 M phosphate buffered saline, pH 7.4.
10. 2% paraformaldehyde in PBS.

Antibody Performance
When assayed by flow cytometric analysis (with a FACScan flow cytometer) using 5 µl of the antibody to stain 1 x 10^6 cells or 100 µl whole blood, 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.

Storage
Store at 2-8 °C. Do not freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

11. Whole blood lysing solution.
12. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **or**
   b. Adjust cell suspension to 1 x 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 x 10^6 cells per tube.
2. Add 5 µl of monoclonal antibody to tube(s) containing cells. Vortex tube gently. Incubate at room temperature (18 – 22 °C) for 30 minutes.
   Proper controls to be included for each sample are:
   a. Autofluorescence control: 5 µl diluent in place of monoclonal antibody.
   b. Negative staining control 1: 5 µl isotype-matched non-specific mouse immunoglobulin (Product No. M5534) at the same concentration as test antibody.
3. After 30 minutes, add 2 ml of diluent to all tubes.
4. Centrifuge cells at 500 x g, for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Repeat washing procedure (steps 4-6).
8. After the second wash, resuspend the cells in 100 µl of the fluorochrome conjugated secondary antibody 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.
9. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 8.
   b. If a mononuclear cell suspension is used, proceed to Step 8.
10. Add 2 ml diluent to all tubes.
11. Wash as in steps 4-6 twice.

12. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used. For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems it may be necessary to incubate the cells in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

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