



# Monoclonal Anti-mouse L-Selectin (CD62L)-Fluorescein

Catalog Number: FAB5761F

Lot Number: LGT02

100 Tests

## Reagent Information

**Carboxyfluorescein-conjugated rat monoclonal anti-mouse CD62L:** Supplied as 75 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 95218

Ig class: rat IgG<sub>2A</sub>

## Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

## Storage

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

## Intended Use

Designed to quantitatively determine the percentage of cells bearing the CD62L cell surface antigen within a population and qualitatively determine its density on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody that binds to the cells expressing CD62L. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing CD62L are fluorescently stained, with the intensity of staining directly proportional to the density of CD62L. Cell surface expression of CD62L is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

## Reagent Preparation

Use as is; no preparation is necessary.

## Sample Preparation

**Peripheral blood cells:** Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Blood cells will require lysis of RBC following the staining procedure.

**Cell Cultures:** Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) are transferred to a 5 mL tube for staining.

**Note:** Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

## Sample Staining

- 1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment 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.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of fluorescein-conjugated anti-mouse CD62L reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unbound anti-mouse CD62L reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
- 6) Resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
- 7) As a control for analysis, cells in a separate tube should be treated with fluorescein-labeled rat IgG<sub>2A</sub> antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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## Background Information

Mouse CD62L, also known as L-Selectin, LECAM, and Ly-22, is a member of the C-type lectin family that is expressed on most peripheral leukocytes (1, 2). CD62L is thought to mediate lymphocyte homing to high endothelial venules as well as the rolling of leukocytes on activated endothelium (3). CD62L is proteolytically cleaved from the cell surface following cellular activation (4, 5). CD62L is a useful marker in identifying naïve and memory T cells (6).

## References

1. Iwabuchi, K. *et al.* (1991) *Immunobiol.* **182**:161.
2. Lewinsohn, D.M. *et al.* (1987) *J. Immunol.* **138**:4313.
3. Spertini, O. *et al.* (1991) *J. Immunol.* **147**:2565.
4. Chen, A. *et al.* (1995) *J. Exp. Med.* **182**:519.
5. Kishimoto, T.K. *et al.* (1989) *Science* **245**:1238.
6. Sprent, J. and D.F. Tough (1994) *Science* **265**:1395.

**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.