Biotin Anti-Rat CD54 (ICAM-1)
Monoclonal Antibody

CL054B
LOT: 0902

DESCRIPTION:

Cedarlane's anti-rat CD54 (ICAM-1) monoclonal antibody recognizes the rat intercellular adhesion molecule-1, designated as CD54. ICAM-1 is a 90kDa adhesion molecule belonging to the superimmunoglobulin family. It is a cell surface ligand of the lymphocyte integrin, LFA-1 (lymphocyte function associated antigen-1) and is known to play an important role in various cell-cell interactions in the immune system. ICAM-1 exists on fibroblasts, epithelial and endothelial cells.

This monoclonal antibody inhibits homotypic aggregation of PHA blasts. Immunoprecipitation analysis shows that the antigen has features identical to those of human ICAM-1. Antigen distribution is in full agreement with that reported with the human ICAM-1.

Applications include immunoprecipitation, flow cytometry analysis and immunohistochemistry (frozen sections) and in vivo and in vitro function blocking (1,2,3,4,5,6).

PRESENTATION:

100 µg biotin conjugated Ig buffered in PBS, pH 7.4, 0.09% sodium azide (NaN₃) and 1% BSA.

STORAGE/STABILITY:

Store at 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles.
SPECIFICATIONS:

Clone: 1A29

Specificity: Rat CD54 (ICAM-1)

Ig Class: Mouse IgG₁

Antibody Concentration: 0.1 mg/ml

FLOW CYTOMETRY ANALYSIS:

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Rat cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10⁷ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10⁶ cells, representing 1 test).
4. To each tube, add ~0.25 µg* of CL054B.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of detection reagent CLCSA1004 (Streptavidin-PE) at a concentration of 1:50.
9. Incubate the tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Continued Overleaf...
Results:

**Tissue Distribution by Flow Cytometry Analysis:**
*(Representative Histogram)*

Rat Strain: Wistar
Cell Concentration: $1 \times 10^6$ cells per test
Antibody Concentration Used: $0.25 \mu g/10^6$ cells
Isotypic Control: Strepavidin-PE (CLCSA1004)

Cell Source: Rat Spleen

**IMMUNOHISTOCHEMISTRY:**

**Method:**

1. Dilute the antibody 1:500 - 1:1000 to stain tissue sections.

2. Cryostat sections should be fixed in cold acetone and incubated with 50-100 µl of diluted antibody/tissue sections.

*N.B. Appropriate control samples should always be included in any labelling studies.*

*For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.*

**REFERENCES:**


**Laboratory Reagent For Research Use Only**

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