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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Anti-Rat CD43
Monoclonal Antibody - Ascites

CL002A
LOT: J1210

DESCRIPTION:

Cedarlane's anti-rat CD43 monoclonal antibody recognizes a monomorphic determinant expressed on rat thymocytes, polymorphonuclear cells, plasma cells and stem cells, but not B lymphocytes or pre-B cells (1,3). The antigen is a heavily glycosylated glycoprotein of apparent molecular weight 95 kDa and has a high content of O-linked carbohydrate structures (3). This major glycoprotein of thymocytes and T lymphocytes is referred to by several names including leukocyte sialoglycoprotein and leukosialin. The carbohydrate structures of leukosialin account for approximately 60% of its weight (2). On thymocytes, this glycoprotein is the main target for binding of peanut lectin (4). This antibody is useful for labelling T but not B lymphocytes and in studies on stem cells since pre-B cells are not labelled while the multipotential stem cell is. It may also be used in analysis of NK cells (5) and in molecular studies in the sialoglycoprotein which it recognizes.

PRESENTATION: 0.5 ml, lyophilized

STORAGE/STABILITY:

Lyophilized form stable at 4°C or -20°C. Reconstitute with 0.5 ml of cold distilled water. After reconstitution, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles. If slight turbidity appears, clarify by centrifugation before use.

For more information or to place an order please contact...

CEDARLANE® LABORATORIES LIMITED
5516 - 8th Line, R.R.#2, Hornby, Ontario, CANADA L0P 1E0

toll free: 1-800-268-5058
in North America
phone: (905) 878-8891 • fax: (905) 878-7800

or visit our website for a list of our international distributors including contact information
website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

SPECIFICATIONS:

Clone: W3/13 HLK

Hybridoma Production:

Immunization: Immunogen: Rat thymocyte membrane
Immunocyte Donor: BALB/C spleen

Fusion Partner: NS1/1

Specificity Rat CD43

Isotype: Mouse IgG1

Presentation: Ascitic Fluid (lyophilized)

FLOW CYTOMETRY ANALYSIS:

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Rat. cell separation media (CL5040).
2. Wash 2 times.
3. Resuspend cells to $1x10^6$ cells in approximately 50 µl Media A in a microcentrifuge tube. (i.e. 50 µl of cells resuspended to $2x10^7$ cells/ml.) (the contents of 1 tube represent 1 test.)
4. To each tube add 50 µl of a 1:5 000-1:10 000 dilution of **CL002A***.
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 ul of secondary antibody **CLCC30201** (FITC Goat anti-mouse IgG (H+L)) at 1:700 dilution.
9. Incubate 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 phosphate buffered saline. (This stains dead cells by intercalating DNA.)

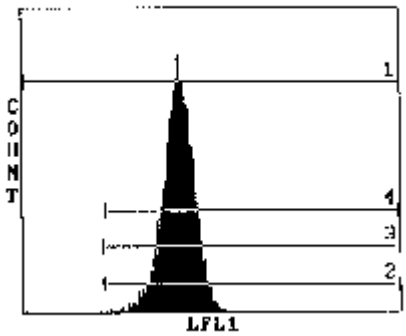
MEDIA

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

B. Phosphate buffered saline (pH 7.2) + 0.5 % bovine serum albumin + sodium azide (100 µl of 2 M sodium azide in 100 mls.)

Rat Strain: Wistar
Cell Concentration: $1x10^6$ cells per test
Antibody Concentration: 1:10 000
IsotypicControl: Mouse IgG1, κ

CELL SOURCE	**PERCENT STAINING**
Thymus	98.5%
Spleen	59.5%
Lymph Node	53.5%



Cell Source: Thymus
Percentage of Cells Stained Above Control: 98.5 %

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.**

STRAIN DISTRIBUTION:

Procedure: As above
Antibody Concentration: 1:5 000
Strains Tested: Lewis, Wistar, ACI, Brown Norway, Fischer 344, Buffalo

Positive: Lewis, Wistar, ACI, BN, Fischer 344, Buffalo
Negative: none

REFERENCES

1. Williams, A.F., Galfre, G. and C. Milstein. (1977) Cell. 14, 633-673. Analysis of cell surfaces by xenogenic myeloma-hybrid antibodies: Differentiation antigens of rat lymphocytes.
2. Dyer, M.J..S. and S.V. Hunt. (1981) J.Exp.Med. 154, 1164-1177. Characterization by surface W3/13 antigen and radiosensitivity.
3. Brown, W.R.A., Barclay, A.N., Sunderland, C.A. and A.F. Williams. (1981) Nature. 289, 456-460. Identification of glycophorin-like molecule at the cell surface of rat thymocytes.
4. Brown, W.R.A. and A.F. Williams. (1982) Immunology. 46, 713-726. Lymphocyte cell surface glycoproteins which bind to soybean and peanut lectins.
5. Cantrell, D.A. Robins, R.A. Brooks, C.G. and R.W. Baldwin. (1982) Immunology. 45, 97-103.
6. Killeen, N., Barclay, A.N., Willis, A.C. and A.F. Williams. (1987) The EMBO J., vol.6, #13, 4029-4034. The sequence of rat leukosialin (W3/13 antigen) reveals a molecule with O-linked glycosylation of one third of its extracellular amino acids.

FOR RESEARCH USE ONLY

(Not to be administered to humans or animals nor used for any drug purpose.)