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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 CD45RA/B
Monoclonal Antibody-Ascites

CL033A
LOT: N6284

DESCRIPTION: Rat Leukocyte Common Antigen has been shown to exist in different forms on different lymphoid cell types. Rat L-CA on thymocytes shows one main band at 180 kDa, T cells show 4 bands at 180, 190, 200 and 220 kDa. CL033A detects a subfraction of the 240 kDa rat L-CA band found only on B lymphocytes. This monoclonal antibody is of particular use in the investigation of the molecular and antigenic heterogeneity of rat L-CA, especially when used in conjunction with other OX antibodies. Four mouse monoclonal antibodies (MRC OX 1, 28, 29 and 30) react with all molecular forms of L-CA and fall into two sets that are non-competitive in binding to L-CA (MRC OX 1, 28, 29 and 30). The antigenic determinants seen by all these antibodies are lost when L-CA is reduced and alkylated. Three antibodies (MRC OX 22, 31, and 32) react selectively with B cells, T cytotoxic cells and about two thirds of T helper cells. OX 22 and OX 31 compete for binding but are non- competitive with OX 32. All of these antibodies bind to a sub-fraction of the 190, 200 and 220 kDa forms of T cell L-CA but not at all to the 180 kDa form of T cells or thymocytes.

This clone is reported to work with frozen and paraffin sections (2).

PRESENTATION: 0.5 ml, lyophilized

STORAGE AND RECONSTITUTION: Store at -20°C or below before reconstitution. Reconstitute with 0.5 ml of cold distilled water. Aliquot and freeze the unused portion in volumes appropriate for single use (to avoid repeated freezing and thawing.) 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

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SPECIFICATIONS:

CLONE: MRC OX-33

HYBRIDOMA PRODUCTION:

IMMUNIZATION:

IMMUNOGEN: Rat purified spleen L-CA
IMMUNOCYTE DONOR: BALB/c Spleen
FUSION PARTNER: NSO/U
SPECIFICITY: Rat CD45RA/B
Ig CLASS: 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 medium (CL5040).
2. Wash 2 times.
3. Resuspend cell to 1 x 10^6 cells in approximately 50 µl Media A in a microcentrifuge tube. (ie. 50 µl of cells resuspended to 2 x 10^7 cells/ml).
4. To each tube add 50 µl of a 1:20,000-1:50,000 dilution of **CL033A**.
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 secondary antibody **CLCC30201** (Goat anti-mouse IgG (H+L)-FITC conjugate) 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

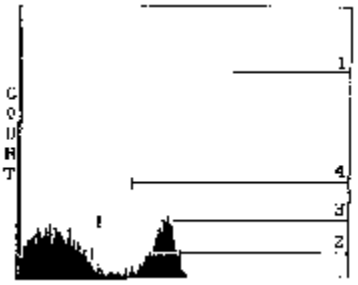
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 ul of 2M sodium azide in 100 mls.)

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

FLOW CYTOMETRIC ANALYSIS

DONOR: Wistar Rat
CELL CONCENTRATION: 1 x 10^6 cells
ANTIBODY CONCENTRATION: 1:40,000
CELL SOURCE: Splenocytes
ISOTYPIC CONTROL: Mouse IgG1, k

CELL SOURCE	PERCENT STAINING
Thymus	1
Spleen	24
Lymph Node	10



LFL1
Cell Source: Spleen
Percentage of cells stained above control: 24%

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: 1:1000
STRAINS TESTED: Lewis, Wistar, ACI, Brown Norway, Buffalo, Fischer 344

POSITIVE: Wistar, Buffalo, Brown Norway, ACI, Fischer 344, Lewis
NEGATIVE: none

REFERENCES:

1. Woolett, G.R., Barclay, A.N. Paklavec, M., and A.G. Williams. (1985) Eur. J. Immunol. 15, 168-173. Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes.

2. Whiteland, J.L et al (1995). Immunohistochemical detection of T cell subsets and other leukocytes in paraffin embedded rat and mouse tissues with monoclonal antibodies .J. Histochem. Cytochem. 43: 313-320.

FOR LABORATORY RESEARCH ONLY

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