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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Biotin Anti-Mouse CD45R, B220 (Ly 5) Monoclonal Antibody

CL8990B
CL8990B-3
LOT: 8041

DESCRIPTION:

Cedarlane's anti-mouse CD45R, B220 (Ly 5) monoclonal antibody reacts with a form of the CD45 antigen on B cells and lytically active subsets of NK cells and non-MHC restricted CTL's [1,2,3,4].

This antibody immunoprecipitates the high molecular weight (220,000 Da) surface molecule of the leukocyte common antigen B220 on B cells [1]. Applications include flow cytometry and immunoprecipitation. Also reacts with human B cells and is reported to work in immunohistochemical applications, both frozen and paraffin sections [5].

PRESENTATION:

100 µg (CL8990B) or 300 µg (CL8990B-3) Biotin conjugated Ig buffered in PBS , 0.02% NaN_3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

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.

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

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SPECIFICATIONS:

Clone: RA3-6B2

Hybridoma Production:

Immunization: Immunogen: Mouse pre-B tumour cells (RAW112)
Donor: Lewis Rat spleen
Fusion Partner: S194/5. XXO.BU-1

Specificity: Mouse CD45R, B220 (Ly 5)

Ig Class: Rat IgG_{2a}

Format: Biotin conjugated Ig buffered in PBS, 0.02% NaN_3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from ascitic fluid via Protein G Chromatography)

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®-M cell separation medium (CL5030).
2. Wash 2 times.
3. Resuspend the cells to a concentration of $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.2-0.5 µg***** of **CL8990B or CL8990B-3** per 10^6 cells.
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 **CLCSA1001** (Streptavidin-FITC) at a 1:500 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
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:

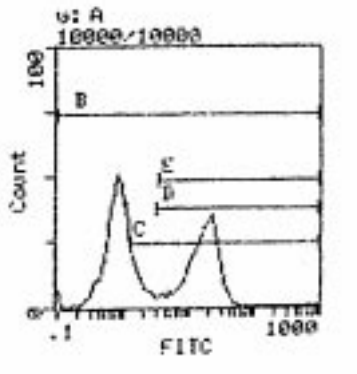
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: C3H/He
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 0.5 µg/10^6 cells
Isotypic Control: Biotin Rat IgG_{2a}

Cell Source	Percentage of cells stained above control:
Thymus	4.4 %
Spleen	43.2%
Lymph Node	21.2%
Human Peripheral Blood Lymphocytes	31.5%



LFL1
Cell Source: Spleen
Percentage of cells stained above control: 43.2%

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 by Flow Cytometry Analysis:

Procedure: see page 2
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 0.5 µg/10^6 cells
Strains Tested: BALB/c, C3H/He, C57BL/6
Positive: BALB/c, C3H/He, C57BL/6
Negative: None

REFERENCES:

1) Coffman, B. 1982. Surface antigen expression and immunoglobulin rearrangement during mouse pre-B cell development. Immunological Rev. 69:5 - 23.

2) Zuhair, K., Ballas, and Rasmussen, W., 1993. Lymphokine-activated killer cells VII. IL-4 induces an NK1.1 + CD8a+b- TCR αβ B220+ lymphokine-activated killer subset.

3) Asensi, V., and Kimeno, K., et al. 1989. Treatment of autoimmune MRL/1pr mice with anti-B220 monoclonal antibody reduces the level of anti-DNA antibodies and lymphadenopathies. Immunology 68:204 - 208.

4) Ballas, A. K., and W. Rasmussen. 1990. Lymphokine-activated killer (LAK) cells. IV. Characterization of murine LAK effector subpopulations, J. Immunol. 144:386.

5) 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.

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