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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

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

CL8990PE
CL8990PE-3
LOT: 8053

DESCRIPTION:

Cedarlane's anti-mouse CD45R, B220 (Ly 5) monoclonal antibody reacts with a form of the CD45 antigen found 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 ⁽¹⁾ on B cells. Applications include flow cytometry and immunoprecipitation. Also reacts with human B cells.

PRESENTATION:

50 µg (CL8990PE) or 300 µg (CL8990PE-3) PE conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

Store at 4°C. **DO NOT FREEZE.** Avoid prolonged exposure to light.

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

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website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

SPECIFICATIONS:

Clone: RA3-6B2

Hybridoma Production:

Immunization: Immunogen: Mouse pre-B tumour cells
(RAW112)
Donor: Lewis rat spleen

Fusion Partner: S 194/5. XXO. BU-1

Specificity: Mouse CD45R, B220 (Ly 5)

Ig Class: Rat IgG_{2a}

Format: R-PE conjugated Ig buffered in PBS, 0.02% NaN₃ 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 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.2-0.1 μ g of **CL8990PE** or **CL8990PE-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. (It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 μ l ice cold Media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15 μ l 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 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:

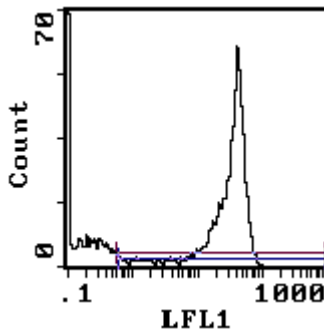
Mouse Strain: BALB/c

Cell Concentration : 1×10^6 cells per tests

Antibody Concentration Used: 0.1 µg/ 10^6 cells,

Isotypic Control: PE-Rat IgG_{2a} (CLCR2A04)

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	0.53%
Spleen	55.7%
Lymph Node	20.0%
Bone Marrow	70.4%



Cell Source: Spleen

Percentage of cells stained above control: 55.7%

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.**

R-Phycoerythrin conjugates are produced under license and protected under Stanford University held patents 4,520,110; 4,542,104; 4,859,582; 5,055,556 (U.S.); 76695 (EPC); 548440 (Australia); 1,179,942 (Canada); and 1,594,827 (Japan).

Strain Distribution by Flow Cytometry Analysis:

Procedure: see page 2

Cell Concentration : 1×10^6 cells per tests

Antibody Concentration Used: $0.1 \mu\text{g}/10^6$ 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 $\alpha\beta$ B220+ lymphokine-activated killer subset.
- 3) Asensi, V., and Kimeno, K., et al. 1989. Treatment of autoimmune MRL/lpr 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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