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PE-Cy5 Anti-Mouse CD117 Monoclonal Antibody

CL8932TC LOT: 03051007

DESCRIPTION:

Cedarlane's monoclonal antibody recognizes CD117 (also known as c-Kit), a tyrosine kinase receptor which is expressed on multipotent hematopoietic stem cells, precursors to B cells and T cells, and myeloerythroid progenerators^{1,3,8}. The interactions of c-Kit and its ligand stem cell factor play a role in the proliferation and differentiation of hematopoietic progenerator cells^{1,8}.

This antibody is suitable for use in flow cytometry and is reported to work in immunoprecipitation..

PRESENTATION:

100 µg PE-Cy5 conjugated Ig buffered in PBS and 0.1% NaN₃ .A highly purified grade of BSA was added as a stabilizer to bring the total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

Store at 4°C. **DO NOT FREEZE**. Avoid prolonged exposure to light. If the reagent it being diluted, it is recommended that only the quantity to be used within one week be diluted. Check label for expiry date.

SPECIFICATIONS:

<u>Clone</u>: 2B8 <u>Specificity</u>: Mouse CD117 (c-kit) <u>Ig Class</u>: Rat IgG_{2b} <u>Format</u>: PE-Cy5 conjugated Ig buffered in PBS and 0.1%NaN₃. <u>Antibody Concentration</u>: 0.2 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 $1x10^6$ cells, representing 1 test).

Continued Overleaf...

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- 4. To each tube, add ~0.25-0.5 µg* of CL8932TC.
- 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 μl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

NOTE: It has been observed that the 2B8 clone conjugated to PE-Cy5 exhibits a degree of non specific staining on myeloid cells in the bone marrow. Preincubation of bone marrow cells with unconjugated anti-mouse CD16/32 (cat# CL9404AP) can reduce the background staining on bone marrow myeloid cells.

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:

<u>Tissue Distribution by Flow Cytometry Analysis</u>: (**Representative Histogram**) Mouse Strain: BALB/c Cell Concentration: 1x10⁶ cells per test Antibody concentration used: 0.25 ug/10⁶ cells Isotypic Control: PE-Cy5 Rat IgG2b (CLCR2B06)



FITC anti-CD11b Cell Source: Bone Marrow

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.

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