PE Anti-Mouse CD28
Monoclonal Antibody

CL8928PE
CL8928PE-3
LOT: 06020806

DESCRIPTION:
Cedarlane’s anti-mouse CD28 antigen monoclonal antibody reacts with CD28 which is weakly expressed by most T cells and NK cells\(^1,2\). Expression of CD28 by thymocytes and splenic T cells is increased after activation\(^1\). CD28 is a co-stimulatory receptor that interacts with CD80 (B7-1) and CD86 (B7-2) on antigen presenting cells\(^3\). The 37.51.1 mAb enhances cytokine production and proliferation of T and NK cells in the presence of certain mitogens\(^1,2\).

This antibody is suitable for use in flow cytometry. It is reported to work immunoprecipitation\(^1\).

PRESENTATION:
50 µg (CL8928PE) or 300 µg (CL8928PE-3) R-PE conjugated Ig buffered in PBS, 0.1% 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. DO NOT FREEZE. Avoid prolonged exposure to light. If the reagent is being diluted, it is recommended that only the quantity to be used within one week be diluted. Check label for expiry date.

SPECIFICATIONS:
Clone: 37.51.1
Specificity: Mouse CD28
Ig Class: Hamster IgG
Antibody Concentration: 0.1 mg/ml

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FLOW CYTOMETRY ANALYSIS:

NOTE: We recommend incubating target cells for 10 minutes in the presence of anti-mouse CD16/32 mAb’s (CL9403AP) to block Fc receptor mediated binding of this antibody to cells.

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 $2 \times 10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1 \times 10^6$ cells, representing 1 test).
4. To each tube, add ~0.25 µg* of CL8928PE or CL8928PE-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 µl 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.

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:
(Representative Dot Plot)
Mouse Strain: C57BL/6
Cell Concentration: $1 \times 10^6$ cells per test
Antibody Concentration Used: 0.25 µg/$10^6$ cells
Isotypic Control: PE Hamster IgG (CLCHM04)

![A. Splenocytes blocked](image)
![B. Splenocytes unblocked](image)

Decreased background staining of 37.51.1 mAb using purified anti-mouse CD16/32 (catalogue # CL9403AP).
Briefly, one million cells from a single cell suspension of C57BL/6 splenocytes were simultaneously stained with 0.25 µg of anti-mouse CD3 conjugated to APC and 0.25 µg of anti-mouse CD28 conjugated to FITC (CL8928F) either with preblocking of Fc receptors for 10 minutes using 0.5 µg of purified anti-mouse CD16/32 (CL9403AP)(Fig. A) or without preblocking Fc receptors (Fig. B). Note the decreased staining of FITC conjugates anti-mouse CD28 on CD3 negative cells when Fc receptors are blocked.

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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