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## PE Anti-Mouse CD86 (B7-2) **Monoclonal Antibody**

**CL8986PE** CL8986PE-3 LOT:

#### **DESCRIPTION:**

This antibody reacts with CD86 (B7-2) which is expressed on resting monocytes, dendritic cells, macrophages and at low levels on B lymphocytes<sup>1</sup>. CD86 plays a role in T cell activation through interactions with CD28 and CTLA-4. Expression of CD86 can be enhanced by activating B cells with certain mitogens.

This antibody is suitable for use in flow cytometry.

#### PRESENTATION:

50 μg (CL8986PE) or 300μg (CL8986PE-3) R-PE conjugated Ig buffered in PBS, 0.1% NaN<sub>3</sub> 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.

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#### **SPECIFICATIONS:**

Clone: RMMP-2

Specificity: Mouse CD86 (B7-2)

Ig Class: Rat IgG<sub>2a</sub>

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  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
- 4. To each tube, add ~1.0 μg\* of **CL8986PE or CL8986PE-3** per 10<sup>6</sup> cells.
- 5. Vortex the tubes to ensure thorough mixing of antibody and cells.
- 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.
- 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:

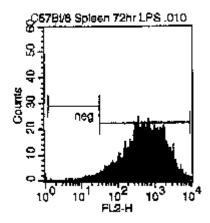
- A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + so-dium azide (100  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).

#### Results:

### Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: C57BL/6

Cell Concentration: 1x10<sup>6</sup> cells per test Antibody Concentration Used: 1.0 µg/10<sup>6</sup> cells Isotypic Control: PE Rat IgG<sub>2</sub> (CLCR2A04)



Cell Source: 72 hour LPS Activated Splenic B Cells Percentage of cells stained above control: 98.1%

# N.B. Appropriate control samples should always be included in any labeling studies.

\* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.

## **References:**

1. Hathcock, K.S., G. Laszlo, C. Pucillo, P. Linsley, and R.J. Hodes. 1994. Comparative analysis of B7-1 and B7-2 co-stimulatory ligands: Expression and function. *J. Exp. Med.* **180**:631-640.

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NWB 03/13/02