

## Product datasheet for **AM31878PU-N**

### Cd80 Rat Monoclonal Antibody [Clone ID: RMMP-1]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	RMMP-1
Applications:	FC
Recommended Dilution:	<b>Flow Cytometry</b> (See Protocols).
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
Specificity:	This antibody recognises the murine CD80 cell surface protein, also known as B7-1.
Formulation:	PBS containing 0.09% Sodium Azide as preservative and EIA grade BSA to bring total protein concentration to 4-5 mg/ml State: Purified State: Liquid purified IgG fraction
Concentration:	lot specific
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD80 antigen
Database Link:	<a href="#">Entrez Gene 12519 Mouse Q00609</a>
Background:	CD80 is a member of the Ig superfamily, along with CD86 (B7-2), participates in T cell costimulation via interactions with CD28 and CTLA-4. CD80 is constitutively expressed on dendritic cells, monocytes, and peritoneal macrophages; and it is inducible on B cells by various means, including activation by LPS, IL-4, and the cross-linking of surface Ig. Expression of CD80 is greatly enhanced on splenic B cells following activation by LPS, with peak expression occurring between 48 and 72 hours. It has been reported that activation of purified B cells with LPS can induce CD80 expression in as few as 18 hours.



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**Synonyms:** CD28LG, CD28LG1, LAB7, BB1, B7.1, B7-1

**Note:** Protocol: **FLOW CYTOMETRY ANALYSIS:**  
**NOTE:** Each lot is tested using freshly harvested mouse splenocytes and mouse splenocytes cultured for 72 hrs with LPS (30 ug/ml).

**Method:**

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of  $2 \times 10^7$  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  $\sim 0.25$   $\mu$ g\* of AM31878PU-N.
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  $\mu$ l of FITC Goat anti-Rat IgG (H+L) secondary antibody at 1:500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes.  
(It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50  $\mu$ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ l 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  $\mu$ 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).