Monoclonal Anti-rat B7-2 (CD86) Antibody

ORDERING INFORMATION

Catalog Number: MAB13401

Clone: 199622

Lot Number: IMV02

Size: 500 μg

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rat B7-2

Immunogen: NS0-derived rrB7-2 extracellular domain

Ig class: mouse IgG_1

Recommended Application:
Neutralization of bioactivity

Other Applications:
Direct ELISA
Western blot

Preparation
This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, NS0-derived, recombinant rat B7-2 (rrB7-2) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. B7-2, also known as CD86, is a type I transmembrane protein belonging to the immunoglobulin superfamily. It is expressed on antigen presenting cells and regulates T cell function through interaction with its receptors, CD28 and CTLA4.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level
< 0.1 EU per 1 μg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 μg/mL.

Storage
Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
This antibody was selected for its ability to neutralize the bioactivity of rat B7-2. In direct ELISAs, this antibody does not cross-react with rrB7-1, rhB7-2, rmB7-2, rmB7-H1, rmB7-H2, rmB7-H3 or rmPD-L.

Applications
Neutralization of Rat B7-2 Bioactivity -The exact concentration of antibody required to neutralize rat B7-2 activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The Neutralization $Dose_{50}$ (ND_{50}) for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response. The ND_{50} for this lot of anti-rat B7-2 antibody was determined to be approximately 0.3 - 1 μg/mL in the presence of 1 μg/mL of rrB7-2, using IL-2 production by the Jurkat E6-1 cell line as an assay. The specific conditions are described in the figure legends.

Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect rat B7-2. The detection limit for rrB7-2 is approximately 20 ng/well.

Western Blot - This antibody can be used at 1 - 2 μg/mL with the appropriate secondary reagents to detect rat B7-2. Using a colorimetric detection system, the detection limit for rrB7-2 is approximately 50 ng/lane under non-reducing and reducing conditions. Chemiluminescent detection with WesternGlo Chemiluminescent Detection Substrate (R&D Systems, Catalog # AR004) will increase sensitivity by 5 to 50 fold. In this application, the use of anti-rat B7-2 monoclonal antibody, R&D Systems, Catalog # MAB1340, is recommended.

Optimal dilutions should be determined by each laboratory for each application

R&D Systems, Inc.
1-800-343-7475

Figure 1

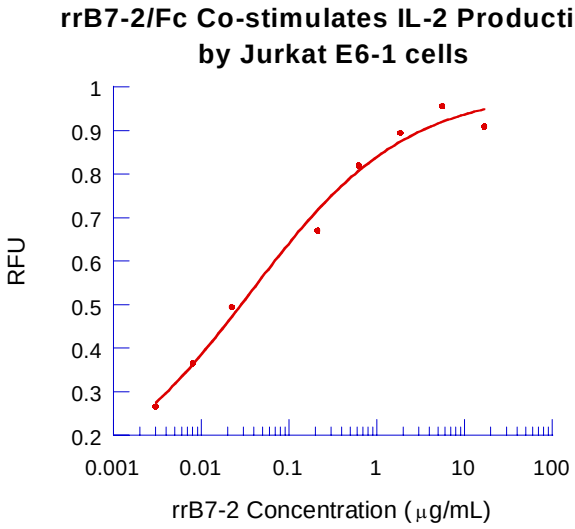

Figure 2

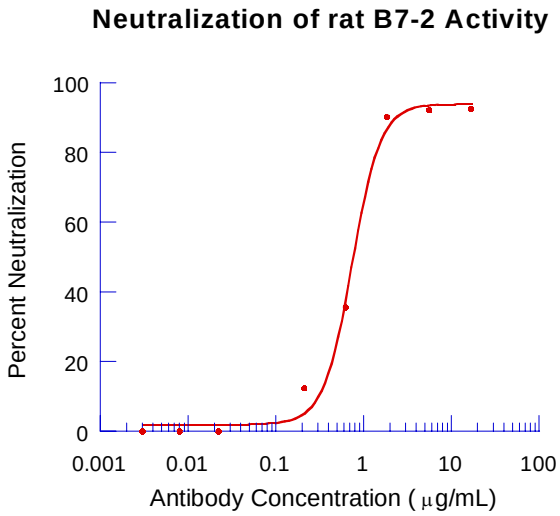

Figure 1
Recombinant rat B7-2/Fc co-stimulates IL-2 production by Jurkat E6-1 cells in the presence of 10 μg/mL PHA (Linsley, P. *et al*., 1990, Proc. Natl. Acad. Sci. USA **87**:5031 - 5035). The ED_{50} for this effect is typically 0.075 - 0.3 μg/mL.

Figure 2
To measure the ability of the antibody to neutralize rat B7-2 mediated activity, various concentrations of anti-B7-2 antibody were incubated with rrB7-2/Fc for 1 hour at 37° C. Following this preincubation period, Jurkat E6-1 cells and PHA were added. The assay mixture, in a total volume of 200 μL/well, containing antibody at the concentrations indicated, rrB7-2/Fc at 1 μg/mL, Jurkat E6-1 cells at 2.5 x 10^4 cells/well and PHA at 10 μg/mL, was incubated at 37° C for 2 - 3 days in a humidified CO_2 incubator. After this incubation, 150 μL of supernatant was collected from each well and tested for human IL-2 levels using a Quantikine IL-2 ELISA kit (R&D Systems, Catalog # D2050). The ND_{50} of the antibody under these conditions is approximately 0.3 - 1 μg/mL.