Anti-human JAM-B/VE-JAM Antibody

ORDERING INFORMATION

Catalog Number: AF1074

Lot Number: GHG01

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human JAM-B

Immunogen: NS0-derived rhJAM-B

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation
Produced in goats immunized with purified, NS0-derived, recombinant junction-associated molecule B (rhJAM-B) extracellular domain. Human JAM-B specific IgG was purified by human JAM-B affinity chromatography. Human JAM-B is a member of the junction adhesion molecules and is alternatively known as VE-JAM.

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 0.1 mg/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 has been selected for its ability to neutralize human JAM-B bioactivity.

Neutralization of Human JAM-B bioactivity
The exact concentration of antibody required to neutralize human JAM-B 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-human JAM-B antibody was determined to be approximately 0.2 - 0.8 μg/mL in the presence of 0.2 μg/mL of rhJAM-B, using the J45.01 cell line. The specific conditions are described in the figure legends.

Additional Applications
Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect human JAM-B. The detection limit for rhJAM-B is approximately 5 ng/lane under non-reducing and reducing conditions.

Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect human JAM-B. The detection limit for rhJAM-B is approximately 0.6 ng/well. In this format, this antibody shows approximately 5% cross-reactivity with rmJAM-B.

Optimal dilutions should be determined by each laboratory for each application.

Figure 1

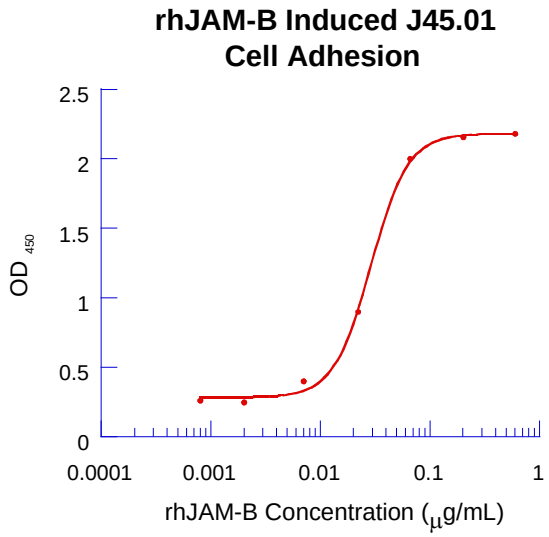

Figure 2

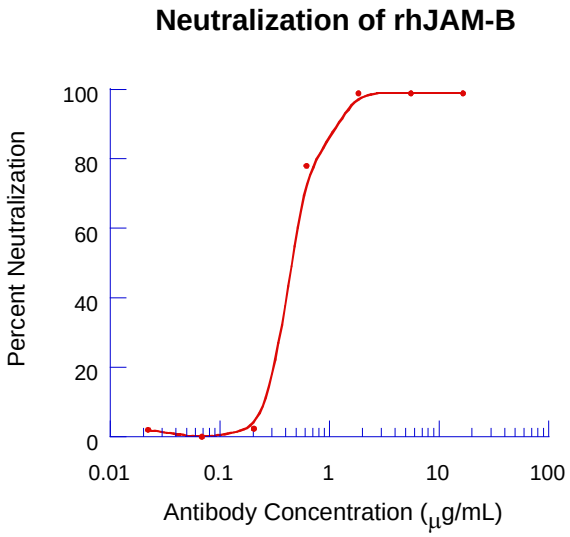

Figure 1
Human JAM-B immobilized onto a microplate previously coated with goat anti-human Fc (10 μg/mL, 50 μL/well) induces J45.01 cell adhesion in a dose-dependent manner (Fong, S., 2002, J. Immunol. **168**:1618 - 1626). 0.2 μg/mL of rhJAM-B immobilized onto a 96 well microplate (100 μL/well) will induce at least 60% adhesion of J45.01 cells added at 2 x 10^5 cells/well.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of rhJAM-B, immobilized rhJAM-B (0.2 μg/mL, 100 μL/well) was incubated with various concentrations of the antibody for 30 minutes at 37° C in a 96 well plate. Following this preincubation period, 50 μL of J45.01 cells at 4 x 10^6 cells/mL were added to each well. The assay plate was incubated at 37° C for 2 hours. At the end of the incubation, non-adherent cells were washed off. Cells attached to the wells were detected by measuring endogenous cellular lysosomal acid phosphatase activity. The ND_{50} of the antibody is approximately 0.2 - 0.8 μg/mL.