EVEC, Inc

COSMO BIO CO., LTD.
Inspiration for Life Science

Antibody Datasheet

Product name
Clostridium difficile Toxin B antibody (4-9A)

Product description
Neutralizing monoclonal antibody to *Clostridium difficile* Toxin B

Catalog Number
EVHM0701-100

Source
Human (recombinant production in CHO-K1)

Isotype
IgG1 Lambda

Form Supplied and Size
Liquid, 100 μg

Concentration and storage buffer
1 mg/mL in Phosphate buffer saline pH 7.4 (containing no preservative)

Storage
Antibody can be kept at 4°C for up to 1 month and should be kept at -20°C or below for long-term storage. To avoid repeated freeze thaw cycles, antibody should be aliquoted before frozen.

Purification
Purified by protein A chromatography. The purity is greater than 95% by SDS-PAGE.

Antigen for Screening
Purified Toxin B from *Clostridium difficile* (VPI10463)

Epitope
Epitope has not been determined.

Applications
ELISA, FCM, Neutralization assay. Other applications have not been tested.

Limitations
This product is to be used for research purposes only.

Background information

Clostridium difficile (*C. difficile*) is the major causative agent of antibiotic associated colitis. Antibacterial agents can alter the intestinal flora and allow *C. difficile* to colonize the gut. *C. difficile* releases toxin A (enterotoxin) and toxin B (cytotoxin), which activate the release of cytokines from human monocytes, and cause diarrhea and colonic inflammation. Both toxins can monoglucosylate and inactivate Rho family small GTPase within target cells, leading to the disruption of vital signaling pathways in the cell.

Immunogen and Recombinant Production Host

This antibody was generated from a healthy individual by a method based on the Epstein-Barr virus transformation of peripheral blood mononuclear cells followed by the isolation of antibody-producing cells. The antibody reactivity for the target antigen was screened by enzyme-linked immunosorbent assay (ELISA) using purified Toxin B from *C. difficile*. The antibody genes were cloned from the antibody-producing cells and introduced into CHO-K1 cells for antibody production.

Application Note

Recommended Starting Dilutions:

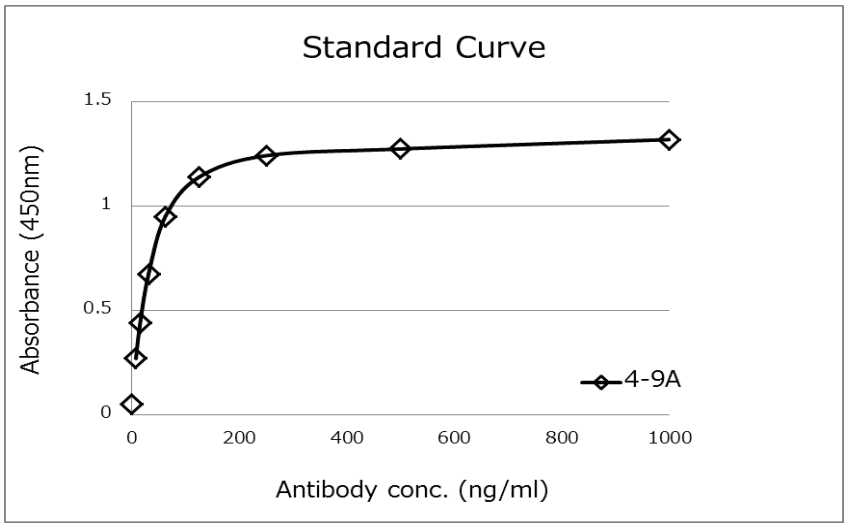
For ELISA: Use at 1:500 – 1:4000
For FCM: Use at 1:500 – 1:4000
Not yet tested in other applications.

The optimal working dilution should be determined experimentally by the end user.

Neutralization assay

This antibody has the ability to neutralize toxin B by cytotoxic assay using IMR-90 cells (see Procedure "Neutralizing assay"). The 50% and 90% inhibitory dose (IC50 and IC90) were calculated as the concentration of the anti-Toxin B (4-9A, IC50 ≥ 47.2 ng/ml and IC90 ≥ 794.2 ng/ml) within the range of inhibition curve. Antibody 4-9A could not completely inhibit Toxin B cytotoxicity towards IMR-90 cells even at 15 μg/ml.

ELISA Results



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NEUTRALIZATION ASSAY USING IMR-90 CELLS

INTRODUCTION

IMR-90 cells were established from normal human lung fibroblast, which have finite proliferation.

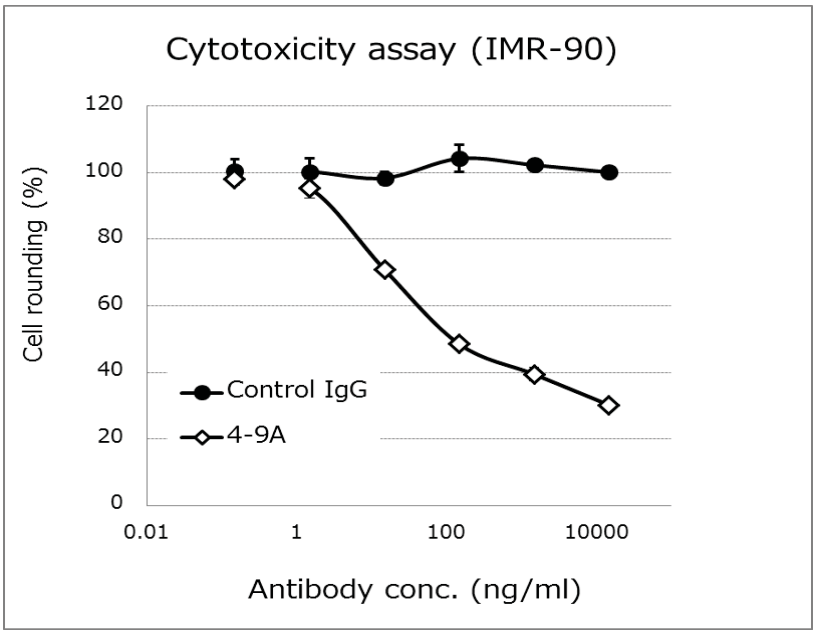
PROCEDURE

CELL MAINTENANCE AND PREPARATION

IMR-90 cells are routinely maintained with 10% FBS-RPMI medium containing antibiotics. For passage, cells are treated with 0.02% EDTA and 0.25% trypsin and then dissociated from the plate. The split ratio should be less than 1/3. IMR-90 cells are not immortal, neutralization assay should be performed using cells that are maintained up to passage number 20.

TOXIN B CYTOTOXICITY ASSAY

1. Plate IMR-90 cells and prepare mono-layer cell plate (eg. in 96-well plate).
2. Next day, prepare antibody (4-9a) serial dilutions with 10% FBS-RPMI medium.
3. Prepare toxin solution with the same medium (effective toxin conc. should be determined before assay).
4. Mix each solution and incubate at 37 °C for 1hr.
5. Remove culture medium from each well and wash each well twice with assay medium.
6. Incubate cells in assay medium at 37 °C, 5% CO_2.
7. Observe cells condition by a microscope.
8. Count all cells ether living and dead (round) cells and calculated cell rounding (%).



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