

Antibody Datasheet

Product name

Human Cytomegalovirus Glycoprotein B antibody (5-7D)

Product description

Complement-independent neutralizing monoclonal antibody to HCMV gB

Catalog Number

EVHM0201-100

Source

Human (recombinant production in CHO-K1)

Clonality and Clone name

Monoclonal, 5-7D

Isotype

IgG1 Kappa

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

Recombinant CMV glycoprotein B (AD2) produced by *E. coli*

Epitope

Epitope has not been determined.

Applications

ELISA, Western Blot, FCM, Neutralization assay.

Limitations

This product is to be used for research purposes only.

Background information

Human Cytomegalovirus (HCMV) is a ubiquitous human beta-herpesvirus that establishes life-long latent infections in human following the primary infection. HCMV infection in the immunocompetent host is clinically silent, although it can cause occasionally an acute febrile illness with features of mononucleosis. HCMV can lead to severe diseases or death in immunocompromised hosts and in congenital infection. HCMV glycoprotein B (gB) is an abundant virion envelop protein that is crucial for the infectivity of CMV. HCMV gB is also one of the most immunogenic virus-encoded proteins, and a significant fraction of virus neutralizing antibodies are directed at CMV gB.

Immunogen and Recombinant Production Host

This antibody was generated from a healthy individual by a method based on 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 recombinant CMV gB. 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 ICC/IF: Use at 1:500 – 1:4000

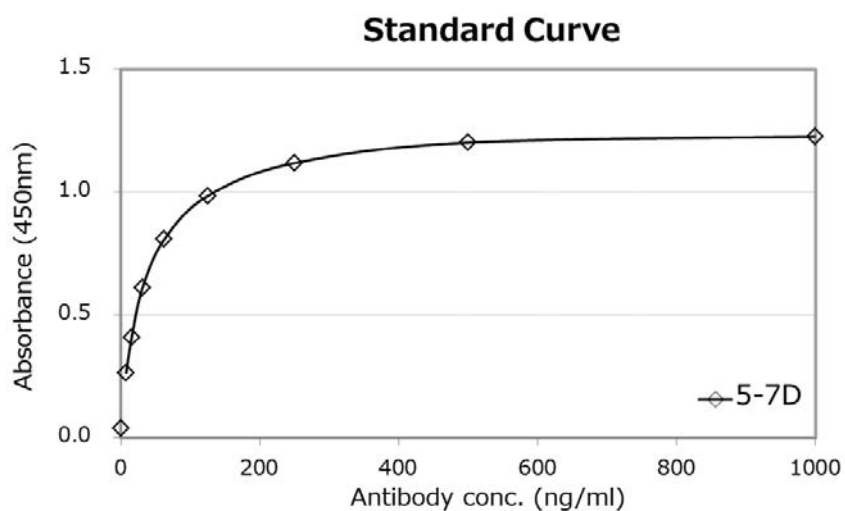
Not yet tested in other applications.

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

Neutralization assay

This antibody has the ability to neutralize hCMV infection using MRC-5 cells (see Procedure "Neutralizing assay using MRC-5 cells"). The 50% and 90% inhibitory dose (IC₅₀) was calculated as the concentration of the anti-hCMV (5-7D, IC₅₀ ≥ 1.98-6.02 µg/ml).

ELISA Results



NEUTRALIZATION ASSAY USING MRC-5 CELLS

INTRODUCTION

MRC-5 cells are derived from normal human lung fibroblast that has finite proliferation.

PROCEDURE

CELL MAINTENANCE AND PREPARATION

MRC-5 cells are routinely maintained with 10% FBS-EMEM medium containing antibiotics. For passage, cells are treated with 0.02% EDTA and 0.25% trypsin and then are dissociated from the plate. The split ratio should be less than 1/3. MRC-5 cells are not infinite, neutralization assay should perform using cells that maintained up to passage number 20.

HUMAN CYTOMEGALOVIRUS NEUTRALIZATION ASSAY

1. Seed MRC-5 cells and prepare monolayer cell plate (i.e. 96-well plate).
2. Next day prepare antibody (5-7D) solutions by ten-fold serial dilution with 10% FBS-EMEM medium.
3. Prepare virus solution (i.e. hCMV, AD169 strain) using the same medium.
4. Mix all components and incubate for 1hr.
5. Add mixture gently onto MRC-5 cells.
6. Incubate cells at 37 °C 5% CO₂ for 20 hrs.
7. Fix cells by 4% paraformaldehyde-PB and stained IE1.
8. Count positive cells and calculate inhibition rate (%).



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