Product Manual

Human Cytomegalovirus Glycoprotein B (HCMV gB) ELISA Kit

Catalog Numbers

VPK-5172 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
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Introduction

The human cytomegalovirus (HCMV) is a widespread pathogen responsible for generally asymptomatic and persistent infections in healthy people. It may, however, cause severe disease in the absence of an effective immune response. HCMV is a member of the herpesviruses. The virion of HCMV consists of a 100-nm diameter icosahedral nucleocapsid containing a 230-kbp, double-stranded linear DNA genome surrounded by a proteinaceous layer defined as the tegument or matrix which, in turn, is enclosed by a lipid bilayer containing a large number of viral glycoproteins. The mature virion particle is 150–200 nm in diameter.

Glycoprotein B (gB), an abundant glycoprotein on the virus envelope and the most highly conserved glycoprotein of the human herpesviruses, is required for virus entry and cell-to-cell spread of the virus.

Cell Biolabs' HCMV gB ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the HCMV gB protein. The kit has detection sensitivity limit of 625 pg /mL HCMV gB. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HCMV samples.

Assay Principle

An anti-HCMV gB monoclonal coating antibody is adsorbed onto a microtiter plate. HCMV gB protein present in the sample or standard binds to the antibody adsorbed on the plate; a FITC-conjugated anti-HCMV gB monoclonal antibody is added and binds to the HCMV gB protein captured by the first antibody. Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-HCMV gB monoclonal antibody. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and a substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of HCMV gB protein present in the sample. The reaction is terminated by the addition of stop solution and absorbance is measured at 450 nm. A standard curve is prepared from the provided HCMV gB protein standard and the sample HCMV gB protein concentration is then determined.

Related Products

1. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV-1 p24 ELISA)
2. VPK-150: QuickTiter™ Hepatitis B Core Antigen (HBcAg) ELISA Kit
3. VPK-156: QuickTiter™ MuLV Core Antigen (MuLV p30) ELISA Kit
4. VPK-5004: QuickTiter™ Hepatitis B Surface Antigen ELISA Kit
5. VPK-5170: RSV Fusion Protein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-HCMV gB Antibody Coated Plate (Part No. 51721B): One strip well 96-well plate.
2. FITC-Conjugated Anti-HCMV gB Monoclonal Antibody (Part No. 51722C): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Viral Lysis Buffer (Part No. 51693B): One 15 mL bottle containing 200 mM Tris, pH 7.5, 1500 mM NaCl, 10% Triton X-100, 1% SDS.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant HCMV gB Protein Standard (Part No. 51723D): One 100 µL vial of 4 µg/mL recombinant human CMV gB in PBS containing BSA.

Materials Not Supplied

1. HCMV Sample: purified virus or unpurified viral supernatant
2. Microcentrifuge
3. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store Recombinant HCMV gB Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Safety Considerations

Remember that your CMV samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing infectious organisms.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-HCMV gB Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of Recombinant HCMV gB Protein Standard in the concentration range of 40 ng/mL – 0.625 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	4 µg/mL Recombinant HCMV gB Standard (µL)	Assay Diluent (µL)	HCMV gB (ng/mL)
1	10	990	40
2	500 of Tube #1	500	20
3	500 of Tube #2	500	10
4	500 of Tube #3	500	5
5	500 of Tube #4	500	2.5
6	500 of Tube #5	500	1.25
7	500 of Tube #6	500	0.625
8	0	500	0

Table 1. Preparation of Recombinant HCMV gB Protein Standard

2. Transfer 225µL of each dilution to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer. Perform the assay as described in Assay Protocol.

CMV Sample Inactivation and Lysis

1. (Optional) Dilute HCMV samples in culture medium. Include culture medium as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer, vortex well. Inactivate HCMV sample at 56ºC for 30 min.
3. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as HCMV lysate.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each HCMV lysate sample, recombinant HCMV gB standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of HCMV lysate or recombinant HCMV gB standard to Anti-HCMV gB Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37ºC for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

6. Add 100 µL of the diluted FITC-Conjugated Anti-HCMV gB Monoclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical HCMV gB ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

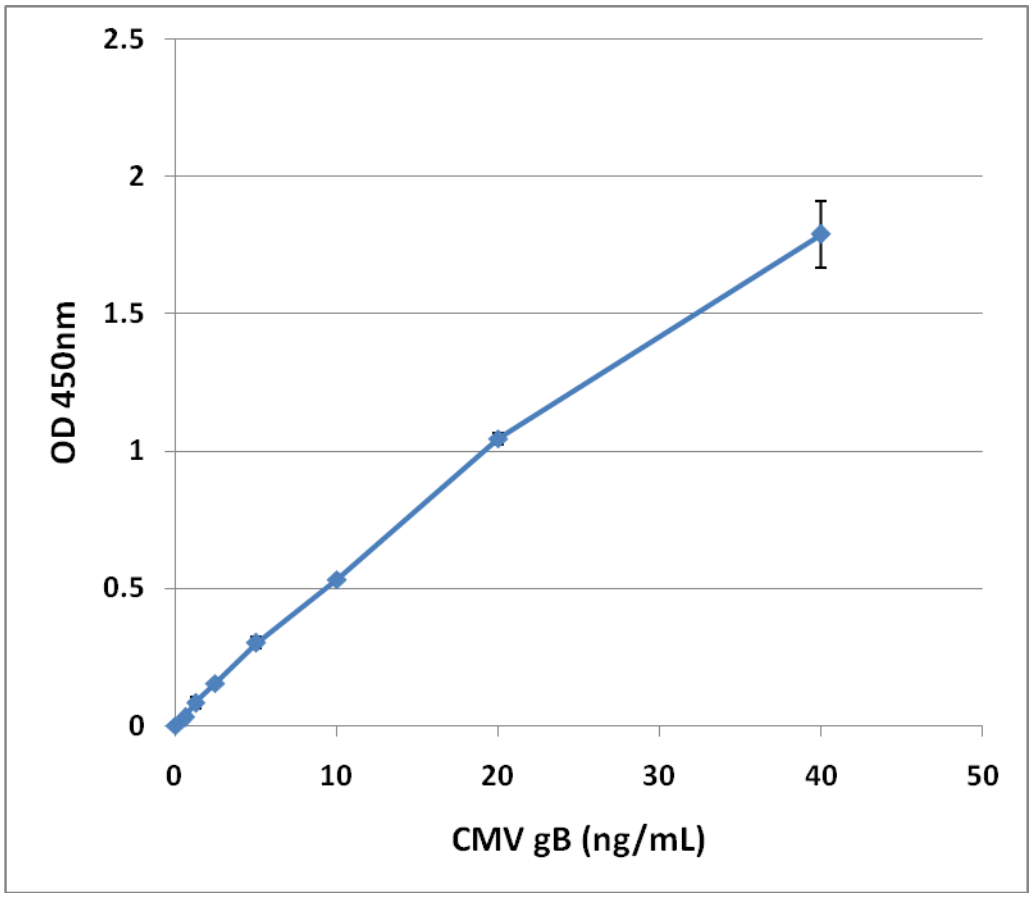

Figure 1: HCMV gB ELISA Standard Curve

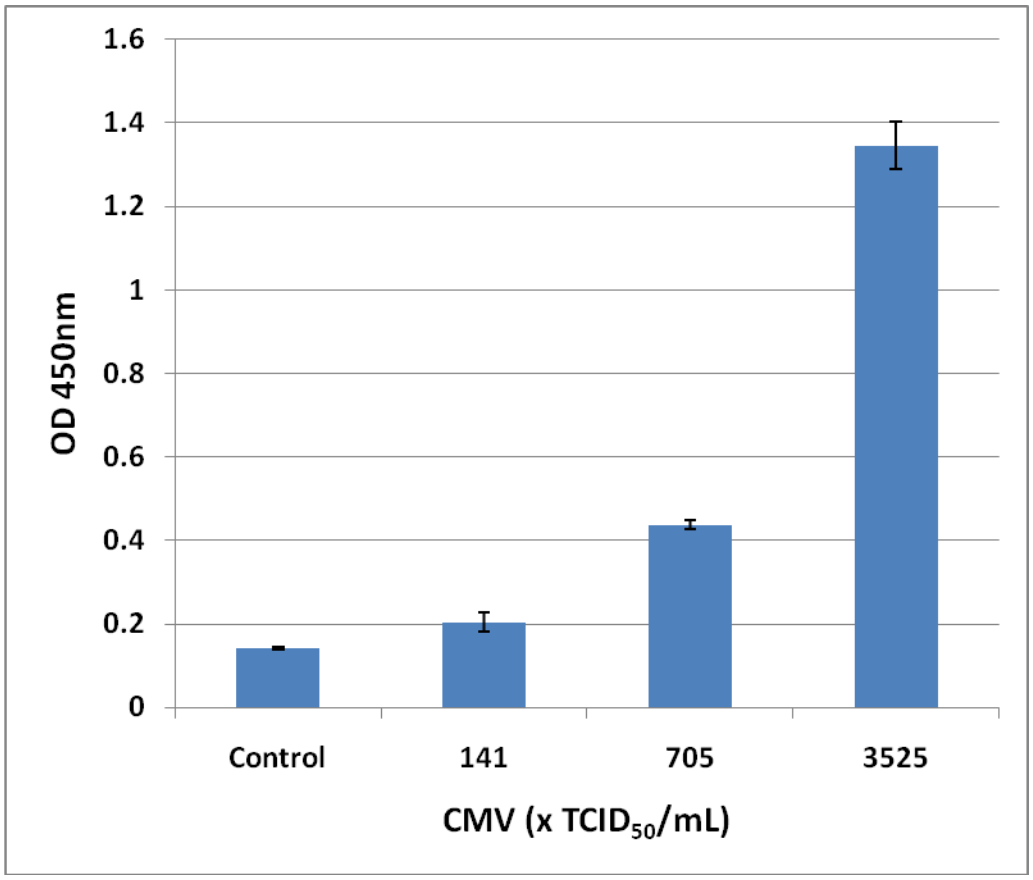

Figure 2: gB in HCMV Culture Fluid. CMV culture fluid (HCMV strain AD-169, 1.41 x 10^5 $TCID_{50}$/mL) was first diluted 20-fold with culture medium, then heat inactivated and lysed in Viral Lysis Buffer. HCMV lysate was subjected to HCMV gB ELISA Kit according to Assay Protocol.

References

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