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Product Manual

# Human Cytomegalovirus Immediate-Early 2 (HCMV IE2) ELISA Kit

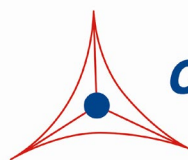
Catalog Number

VPK-5197

96 assays

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **Introduction**

HCMV is a member of the herpes virus family. 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.

HCMV major immediate early (MIE) gene locus produces immediate-early 1 (IE1) and 2 (IE2) gene products. The IE2 protein exists as a p86 isoform and several smaller isoforms. IE2 can bind to a repressor sequence within MIE to downregulate IE1 and IE2 production. IE2 is also involved in activation of gene expression for cell cycle progression but can also halt cell cycle progression. Furthermore, IE2 can bind to a number of other host proteins including p21, Cdt1, basal transcription factors, histone acetylases, histone deacetylases, histone methyl transferases, SUMO1, mdm2, and sp1.

Cell Biolabs' HCMV IE2 ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the HCMV IE2 protein. The kit has a detection sensitivity limit of 3.9 ng/mL HCMV IE2. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HCMV samples.

## **Assay Principle**

HCMV IE2 protein present in the sample or standard binds to the Anti-HCMV IE2 Antibody Coated Plate. Next, a biotin-conjugated anti-HCMV IE2 polyclonal antibody is added and binds to the HCMV IE2 protein captured by the first antibody. Following incubation and wash steps, an HRP-streptavidin conjugate is added and binds to the biotin-conjugated anti-IE2 antibody. Unbound HRP-streptavidin conjugate 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 IE2 protein present in each sample. The reaction is terminated by addition of Stop Solution, and the absorbance is measured at 450 nm. The HCMV IE2 protein concentration in unknown samples is determined by comparing against a standard curve generated from known concentrations of the protein.

## **Related Products**

1. VPK-150: QuickTiter™ Hepatitis B Core Antigen (HBcAg) ELISA Kit
2. VPK-151: QuickTiter™ Hepatitis C Core Antigen (HCcAg) ELISA Kit
3. VPK-5170: RSV Fusion Protein ELISA Kit
4. VPK-5171: RSV Nucleoprotein ELISA Kit
5. VPK-5172: Human Cytomegalovirus Glycoprotein B (HCMV gB) ELISA Kit

## **Kit Components**

### **Box 1 (shipped at room temperature)**

1. Anti-HCMV IE2 Antibody Coated Plate (Part No. 51971B): One strip well 96-well plate.
2. Biotinylated Anti-HCMV IE2 Antibody (Part No. 51972C): One 10  $\mu$ L vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20  $\mu$ L 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. HCMV IE2 Protein Standard (Part No. 51973D): One 50  $\mu$ L vial of 25  $\mu$ g/mL recombinant human CMV IE2.

## **Materials Not Supplied**

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

## **Storage**

Upon receiving, aliquot and store Recombinant HCMV IE2 Standard at  $-80^{\circ}\text{C}$  and avoid freeze/thaw. Store the Biotinylated Anti-HCMV IE2 at  $-20^{\circ}\text{C}$ . Store the 10X Viral Lysis Buffer at room temperature. Store all other components at  $4^{\circ}\text{C}$ .

## **Safety Considerations**

Remember that your HCMV 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.
- Biotinylated Anti-HCMV IE2 Antibody and Streptavidin Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-HCMV IE2 Antibody and Streptavidin Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

## **Preparation of Standard Curve**

1. Prepare a dilution series of HCMV IE2 Protein Standard in the concentration range of 250 ng/mL – 3.9 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

<b>Standard Tubes</b>	<b>25 µg/mL HCMV IE2 Standard (µL)</b>	<b>Assay Diluent (µL)</b>	<b>HCMV IE2 (ng/mL)</b>
1	5	495	250
2	250 of Tube #1	250	125
3	250 of Tube #2	250	62.5
4	250 of Tube #3	250	31.3
5	250 of Tube #4	250	15.6
6	250 of Tube #5	250	7.8
7	250 of Tube #6	250	3.9
8	0	250	0

**Table 1. Preparation of HCMV IE2 Protein Standard**

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

## **HCMV 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 56°C for 30 min.
3. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as HCMV lysate.

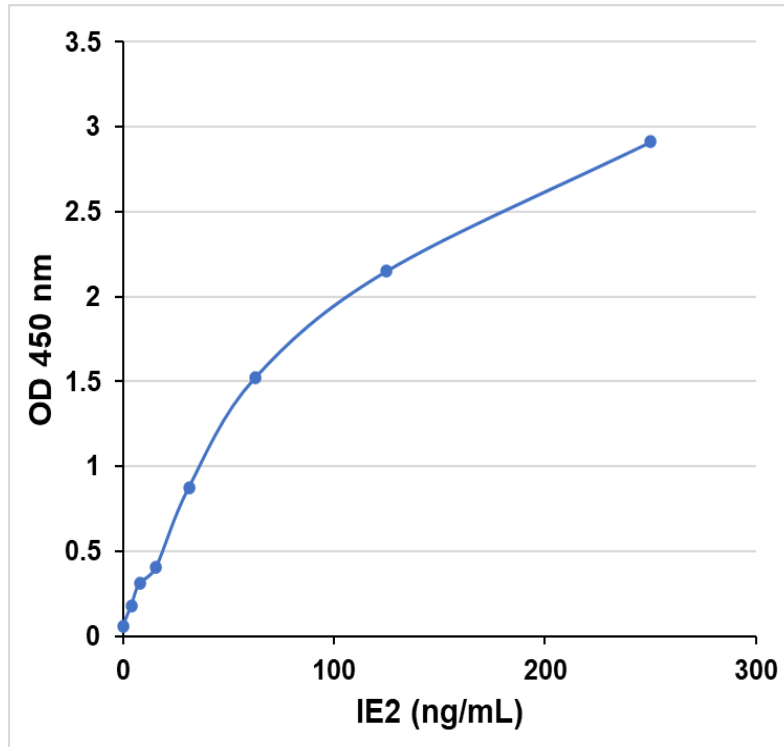
## **Assay Protocol**

1. Prepare and mix all reagents thoroughly before use.
2. Each HCMV lysate sample, HCMV IE2 standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of the HCMV lysate or HCMV IE2 standard to the Anti-HCMV IE2 Antibody Coated Plate.
4. Incubate at room temperature for 1 hour.
5. Remove the solution and wash microwell strips 3 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 an absorbent pad or paper towel to remove excess 1X Wash Buffer.

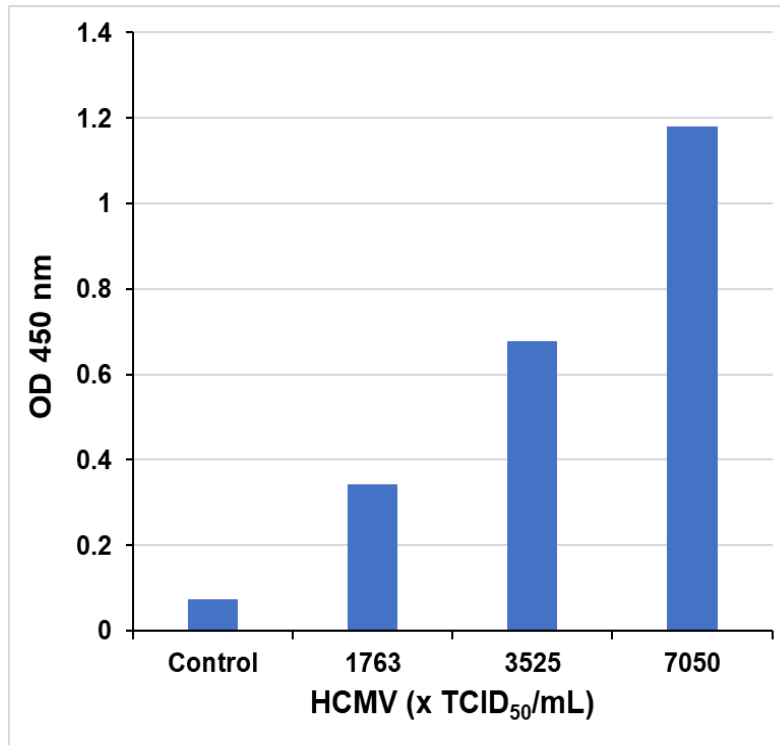
6. Add 100  $\mu$ L of the diluted Biotinylated Anti-HCMV IE2 Antibody to each well.
7. Incubate at room temperature for 1 hour on an orbital shaker.
8. Remove the solution and wash the strip wells 3 times according to step 5 above.
9. Add 100  $\mu$ L of the diluted Streptavidin-Enzyme Conjugate to each well.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove the solution and wash microwell strips 3 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100  $\mu$ L 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  $\mu$ L 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

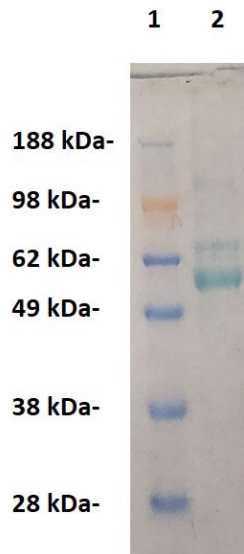
The following figures demonstrate typical HCMV IE2 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: HCMV IE2 ELISA Standard Curve**



**Figure 2: IE2 in HCMV Culture Fluid.** HCMV culture fluid ( $1.41 \times 10^5$  TCID<sub>50</sub>/mL) was first diluted 10-fold with culture medium, then heat inactivated and lysed in Viral Lysis Buffer. HCMV lysate was tested according to the Assay Protocol.



**Figure 3: Purification of Recombinant HCMV IE2 C-terminal (IE2CT) protein.** Lane 1: MW standard; Lane 2: Purified recombinant HCMV IE2CT. The purified recombinant HCMV IE2CT protein was used as the immunogen to produce the ELISA kit antibodies.

MASHAGIDSSSTGPTLLTTHSCSVSSAPLNKPTPTSVAVTNTPLPGASATPELSPRKKPRKTTRPFKVI  
IKPPVPPAPIMLPLIKQEDIKPEPDFTIQYRNKI IDTAGCIVISDSEEEQGEEVETRGATASSPSTGS  
GTPRVTSPTHPLSQMNHPPPLPDPLGRPDEDSSSSSSSSSCSSASDSESESEEMKCSSGGGASVTSSHHG  
RGGFGGAASSLLSCGHQSSGGASTGPRKKKSKRI SELDNEKVRNIMKDKNTPFCTPNVQTRRGRVKI  
DEVSRMFRNTNRSLEYKNLPFTIPSMHQVLDEAIKACKTMQVNNKGIQI IYTRNHEVKSEVDAVRCRL  
GTMCNLALSTPFLMEHTMPVTHPPEVAQRTADACNEGVKAAWLSKELHPTHQLCPRSSDYRNMI IHAAT  
PVDLLGALNLCPLMQKFPKQVMVRI FSTNQGGMFLPIYETA AKAYAVGQFEQPTETPPEDLDTL SLA  
IEAAIQDLRNKSQAAA LEHHHHHH

**Figure 4: HCMV IE2CT Protein Sequence.** Recombinant HCMV IE2CT protein sequence is underlined.

## **References**

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## **Warranty**

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