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Cytomegalovirus (CMV) IgG ELISA Kit

01/16

(Catalog # K4150-100, 100 assays; Store at 4°C)

I. Introduction:

Cytomegalovirus (CMV) is a member of the herpes group of viruses. Most adults and children who catch CMV have no symptoms and are not harmed by the virus. CMV infection is of clinical significance primarily in pregnant women, newborn infants with possible congenital infection, immunosuppressed transplant patients and individuals with AIDS. CMV is so prevalent as over 60% of people catch the infection at some time in their lives. Significant increases in CMV IgG antibody by ELISA suggest recent infection or reactivation of a latent CMV infection. ELISA can detect CMV IgM antibody in both primary CMV infections (93-100%) and in reactivated infection (40%). An IgM response may be reduced or absent in immunocompromised patients with active infection. In transplant patients the CMV infection can be associated with higher morbidity and mortality.

II. Application:

Detecting IgG antibody of Cytomegalovirus (CMV) in human. For research use only.

III. Specificity:

Human

IV. Sample Type:

Serum or plasma

V. Kit Contents:

Components	K4150-100	Part No.
Microwells coated with CMV antigen	12 strips x 8 wells	K4150-100-1
Sample Diluent	22 ml	K4150-100-2
Calibrator	1 ml	K4150-100-3
Positive Control	1 ml	K4150-100-4
Negative Control	1 ml	K4150-100-5
Enzyme conjugate	12 ml	K4150-100-6
TMB Substrate	12 ml	K4150-100-7
Stop Solution	12 ml	K4150-100-8
Wash concentrate (20X)	25 ml	K4150-100-9

VI. User Supplied Reagents and Equipment:

- Distilled or deionized water
- Adjustable Precision pipettes
- Disposable pipette tips
- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper

VII. Storage Conditions:

- Kit can be used within one year if stored properly at 4°C.
- Store 1X Was buffer at room temperature afterward dilution.
- Keep microwells sealed in a dry bag with desiccants.
- Avoid expose test reagents to heat, sun or strong light.

VIII. Sample and Reagent Preparation:

- Collect blood specimens and separate the serum.
  Note: Specimens may be refrigerated at 2-8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.
  Lipemic or hemolyzed samples may cause erroneous results.
- Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature afterward.

IX. Assay Protocol:

Precaution:

- Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- Bring all specimens and kit reagents to room temperature and gently mix 30 minutes before the assay.

Assay Procedure:

1. Place the desired number of coated strips into the holder
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples by adding 10 µl of the sample to 200 µl of **sample diluent**. Mix well.

FOR RESEARCH USE ONLY! Not to be used on humans.

3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel to remove residual wash buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel to remove residual wash buffer.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

X. Result Interpretation:

Calculation:

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the **cut-off value**: Calibrator OD x Calibrator Factor (CF).
3. Calculate the **Antibody (Ab) Index**: Dividing the O.D. value of each sample by cut-off value.
   Note: Background subtraction for each reading is optional for calculating the sample Cortisol concentration, and will not change the final results.

<< Example >>

Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

Quality Control:

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than **0.25**
2. The Ab index for Negative control should be less than **0.9**
3. The Ab Index for Positive control should be greater than **1.2**

Final Result Interpretation:

Ab Index	Result
< 0.9	No detectable antibody
0.9 – 1.1	Borderline positive
> 1.1	Detectable antibody

XI. RELATED PRODUCTS:

- Cytomegalovirus (CMV) IgM ELISA (Cat. No. K4151-100)
- Human CellExp™ HVEM/TNFRSF14, human recombinant (Cat. No. 7466-20, -100)
- Ganciclovir (Cat. No. 1918-50, -250, -1000)
- BAY 57-1293 (Cat. No. 2556-5, -25)
- USP7 Antibody (Cat. No. 3747-100)