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Product Information

Cytomegalovirus (CMV) IgG ELISA kit

Catalog Number: EA100936
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The CMV IgG ELISA Kit is intended for the detection of IgG antibody to Cytomegalovirus (CMV) in human serum or plasma.

Background

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.

Principle of the Test

Diluted patient serum is added to wells coated with purified antigen IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with CMV antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: 1 Vial (ready to use)	1 ml
4. Positive Control: 1 vial (ready to use)	1 ml
5. Negative Control: 1 vial (ready to use	1 ml
6. Enzyme conjugate: 1 bottle (ready to use)	12 ml
7. TMB Substrate: 1 bottle (ready to use)	12 ml
8. Stop Solution: 1 bottle (ready to use)	12 ml

9. Wash concentrate 20X: 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation

1. Collect blood specimens and separate the serum.
2. 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.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl 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.
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
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.

Calculation of Results

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 Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of a Standard Curve

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. If the O.D. of the Calibrator should be greater than 0.250.
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.

Interpretation

The following is intended as a guide to interpretation of CMV IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

- **Antibody Index Interpretation**
  - <0.9 No detectable antibody to CMV IgG by ELISA
  - 0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
  - >1.1 Detectable antibody to CMV IgG by ELISA

References

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4. Gratacap-Cavallier B; Bosson JL; Morand P; Dutertre N; Chanzy B; Jouk PS; Vandekerckhove C; Cart-Lamy P; Seigneurin JM. Cytomegalovirus seroprevalence in French pregnant women: parity and place of birth as major predictive factors. Eur J Epidemiol 1998;14(2):147-52.
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6. Rosenthal SL; Stanberry LR; Biro FM; Slaoui M; Francotte M; Koutsoukos M; Hayes M; Bernstein DI. Seroprevalence of herpes simplex virus types 1 and 2 and cytomegalovirus in adolescents. Clin Infect Dis 1997;24(2):135-9.