

Instructions for use  
**CMV IgG ELISA Kit**  
Qualitative/semi-quantitative assay for anti-CMV IgG antibodies  
Product code GD84  
96 tests  
**For *in vitro* research use only**

140108

### 1. Intended use

The CMV IgG kit is a rapid ELISA designed for the semi-quantitative or qualitative detection of IgG antibodies to cytomegalovirus (CMV) in human serum or plasma. The test kit is for research use only.

### 2. Introduction

CMV infections can be classified as congenital (acquired before birth), perinatal (acquired at birth) or postnatal (acquired after birth). Some 95% of newborn infants congenitally infected with CMV exhibit no clinically overt disease at birth. Disease in the remaining 5% can be severe and may result in neurological damage in survivors or death early after birth.

The prognosis for congenitally infected infants who are asymptomatic at birth is variable. Many subsequently develop hearing loss and varying degrees of mental retardation and central nervous system disorders. Surveys show the incidence of congenital CMV infection to be between 0.5 – 2.5%.

Perinatal infected infants start shedding the virus 3 –12 weeks after delivery and generally remain asymptomatic. Postnatal CMV infections are usually asymptomatic and are acquired through close contact with individuals who are shedding the virus. A small percentage of individuals develop a negative heterophile-antibody infectious mononucleosis syndrome characterised by fever, lethargy and atypical lymphocytosis.

In immunocompromised patients e.g. allograft recipients, cancer patients and AIDS patients, CMV infections occur frequently, often from re-activation of latent infection, and may be life-threatening. CMV infections may occur following blood transfusions. Most transfusion acquired CMV infections are either sub-clinical or characterised by CMV mononucleosis. However, in immunocompromised patients, considerable morbidity and mortality can result from a transfusion-acquired CMV infection.

Serologic tests which measure IgG antibodies to CMV can aid in the diagnosis of CMV infection when paired acute and convalescent sera are tested simultaneously and seroconversion or a significant rise in titre can be demonstrated. Also serologic testing of blood transfusion donors and recipients can help prevent transfusion-acquired infection.

### 3. Principle of the test

Diluted serum or plasma specimens (1:100) are incubated for 20 minutes to allow specific antibodies to CMV to bind to the antigen-coated wells. After washing away unbound antibodies and other serum constituents, CMV specific IgG is detected using rabbit anti-human IgG conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate is removed by washing, and TMB enzyme substrate is added for 10 minutes. A blue colour develops if antibodies to CMV are present. Addition of stop solution gives a yellow colour and the optical densities of controls, the standard(s) and samples are measured using a microplate reader.

### 4. Materials included in the Kit

- **Microplate** 96 wells in 12 X 8 break-apart strips, pre-coated with inactivated CMV antigen.
- **Reagent 1:** Sample diluent concentrate, 10ml, (blue). Dilute before use.
- **Reagent 2:** Wash buffer concentrate (X 10), 100 ml. Dilute before use.
- **Reagent 3:** Conjugate (peroxidase conjugated rabbit anti-human IgG), 12 ml, (red). Ready to use
- **Reagent 4:** TMB Substrate, 12 ml. Ready to use
- **Reagent 5:** Stop solution, 12 ml. Ready to use
- **Standards:** 3 IU/ml (yellow), 10 IU/ml & 30 IU/ml (blue) (calibrated against the proposed International Standard for anti-CMV Ig). Ready to use.
- **Positive control:** (20 IU/ml) (red), 1ml liquid. Ready to use.
- **Negative control:** (green), 1ml liquid. Ready to use.

### 5. Other equipment required

10mm X 60mm tubes for dilution, pipettes 10µl, 100µl, 1000µl; repeating dispenser 100µl, microplate reader with 450nm filter, microplate washing device, distilled or de-ionised water, general laboratory apparatus.

### 6. Storage and precautions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiry date if less than three months). It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiry date.

The assay standards and controls are manufactured from dilute non-infectious human serum. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The stop solution contains 0.25M sulphuric acid.

### 7. Samples

Only freshly drawn and properly refrigerated sera or plasma should be used in this assay. Avoid haemolysed, lipemic or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store test sera at -20°C. Avoid multiple freeze-thaw cycles.

### 8. Method

Ensure that all materials are at room temperature before beginning the procedure. We recommend that the standards and the controls are always run in duplicate. Samples may be run singly or in duplicate.

1. Assemble the number of strips required for the assay.
2. The sample diluent X15 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength diluent for the assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2 – 8°C. Prepare working strength sample diluent (Reagent 1) by diluting it 1:14 in distilled/de-ionised water.
3. Dilute patient samples 1:100 (e.g. 5µl serum plus 0.5 ml diluent). It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.
4. For qualitative determinations, dispense 100 µl of the negative control, the 3 IU/ml standard, the positive control and the diluted patient sample into the wells. For semi-quantitative determinations, use sample diluent as 0 IU/ml and dispense in addition the 10 IU/ml and the 30 IU/ml standards.
5. Incubate for **20** minutes at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.
6. Dilute the Wash Buffer (**Reagent 2**) 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water. The diluted wash buffer is stable for two months at 2 - 8°C.
7. After 20 minutes, decant or aspirate the well contents and wash the wells 3 times using an automatic plate washer or the manual wash procedure (see below). Careful washing is the key to good results. Blot the wells on absorbent paper before proceeding. **Do not allow the wells to dry out.**

#### Manual Wash Procedure:

Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process two more times.

8. Dispense 100µl of Conjugate (**Reagent 3**) into each well. This reagent is colour coded red. Keep all pipettes and other equipment used for Conjugate completely separate from the substrate reagent! Incubate the wells for **20** minutes at room temperature.
9. After 20 minutes, discard the well contents and carefully wash the wells with wash buffer. Repeat the washing stage three more times. Ensure that the wells are completely washed. Blot the microplate on absorbent paper to remove final drops of wash fluid. **Do not allow the wells to dry out.**
10. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (**Reagent 4**) into each well. Incubate the plate for **10** minutes.
11. Add 100µl of Stop Solution (**Reagent 5**) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
12. Read the optical density in a microplate reader within 10 minutes.

### 9. Quality control

The expected optical density values for the negative and positive controls and the standards are given on the certificate included in the kit.

### 10. Interpretation

#### Qualitative determinations

Negative samples: OD < OD of 3 IU/ml standard  
Positive samples: OD ≥ OD of 3 IU/ml standard

#### Semi-quantitative determinations

Plot the optical densities of the standards against their concentrations and draw a line through the points. Read the concentrations of the unknowns from this graph. Concentrations below 3 IU/ml are considered negative for anti-CMV IgG. Values above 3 IU/ml are regarded as positive. Values above 30 IU/ml should be re-assayed at a higher dilution.

1. A negative result indicates no current or previous infection with CMV. Such individuals are presumed to be susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgG antibody. If a primary infection is suspected, another specimen should be taken in 8-14 days and tested concurrently in the same assay with original specimen to look for seroconversion.
2. A positive result indicates a current or previous infection with CMV.
3. To evaluate acute and convalescent sera, both samples must be tested in the same assay. If the acute specimen is negative and the convalescent specimen is positive, seroconversion has taken place and a primary CMV infection is indicated.

### 11. Limitations

1. The presence of IgG antibodies to CMV does not necessarily assure protection from future infection with CMV.
2. The antibody titre of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
3. Test results for demonstration of seroconversion should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
4. A positive CMV IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. However, a negative test for IgG antibody in the neonate may help exclude congenital infection. The most definitive diagnosis of active CMV infection requires viral isolation.
5. Specimens containing antibodies to nuclear antigens may give false positive results.

### 12. Expected Values

The incidence of CMV infection varies with age, geographical location, and socio-economic status.

### 13. Performance characteristics

#### Comparative study:

The Genesis Diagnostics CMV IgG kit was compared with another commercially available ELISA procedure for the detection of IgG antibodies to CMV. The Genesis kit showed 100% agreement with the other ELISA. The results are summarised below.

Comparative Study (n=55)	Reference CMV IgG ELISA kit	
	+	-
Genesis Diagnostics CMV IgG kit	27	0
	0	28

### 14. Assay characteristics

Within Assay Imprecision < 12%  
Between Assay Imprecision < 12%

#### **Method Summary**

- Dilute sera 1:100 with sample diluent (**Reagent 1**)
- Dispense 100µl of standard as required
- Incubate for **20** minutes at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate (**Reagent 3**) into each well
- Incubate at room temperature for **20** minutes
- *Wash the wells four times*
- Add 100µl of TMB Substrate (**Reagent 4**) to each well
- Incubate at room temperature for **10** minutes
- Add 100µl Stop Solution (**Reagent 5**) to each well
- Read the optical density at 450nm

#### **Further Reading**

Drew WL: Diagnosis of cytomegalovirus infection. Rev Infect Dis 10:5468-5475, 1988

Booth JC *et al*: Comparison of enzyme-linked immunosorbent assay, radioimmunoassay, complement fixation, anticomplement immunofluorescence and passive agglutination techniques for detecting cytomegalovirus IgG antibody. J Clin Pathol 35: 1345-1348, 1982

Dylewski JS *et al*: Large scale serological screening for cytomegalovirus antibodies in homosexual males by enzyme-linked immunosorbent assay. J Clin Micro 19: 200-203, 1984