Cytomegalovirus IgG ELISA Kit

**Cat.No: DEIA326**
**Lot. No. (See product label)**

### Intended Use

Enzyme immunoassay for the quantitative determination of IgG antibodies against Cytomegalovirus in human serum and plasma.

### General Description

Cytomegaly is caused by a viral infection, which shows an inapparent progression, however leads to serious symptoms with immune-compromised patients and newborns.

The cytomegaly virus (CMV) with a particle diameter of 100 nm (inclusive envelope the virion measures 200 nm) belongs to the family of herpesviridae like Herpes 1 and Herpes 2. The transmission is effected via saliva and by long-term intimate contacts. The incubation time is 2-10 days for primary infections.

Cytomegaly appears often during the gravidity, and per year up to 5% of the pregnant women show clinical signs of the disease. In the early phase the illness frequently remains undetected, and the affected women themselves show no symptoms. When however newborns are infected (1% of the births), these show in 10% of the cases disease symptoms with late damages like retinitis, ulcer of the mucous membrane, viral pneumonia, meningitis, microencephaly, hydrocephalus and skin ulcerations. The incidence of women capable of childbearing is estimated to be 65%, so that an assessment of the immune status before pregnancy is mandatory. After a negative result, a control check is recommended in the 20th week of gestation.

Besides the symptomatic treatment a specific therapy with phosphonoformate and ganciclovir is possible. A vaccination does not exist, so that in the case of an infection only passive CMV immunoglobulins are administered.

Together with the differential assessment of the above mentioned symptoms, the diagnosis is performed clinically in tissue samples via the so-called “owl eyes”. The following laboratory methods are available: CF and ELISA, determination of virus nucleic acid (PCR) as well as virus isolation (cell culture). Serologically, IgG and IgM antibodies can be detected and quantitated by the ELISA technique. Also the measurement of the avidity of the IgG molecules is appropriate.

### Principle Of The Test

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgG. After the substrate reaction the intensity of the color developed is proportional to the amount of IgG-specific antibodies detected. Results of samples can be determined directly using the standard curve.

### Reagents And Materials Provided

1. **Microtiter Plate (MTP):** 1 x 12 x 8.
   Break apart strips. Coated with specific antigen.

2. **Enzyme Conjugate IgG (ENZCONJ IgG):** 1 x 15 mL.

3. **Standard A-D (CAL A-D):** 4 x 2 mL.
   1; 10; 30; 90; U/mL. Ready to use.
   Standard A = Negative Control
   Standard B = Cut-Off Control
   Standard C = Weakly Positive Control
   Standard D = Positive Control
   Contains: IgG antibodies against Cytomegalovirus, Human serum, PBS, stabilizers.
4. **Diluent Buffer (DILBUF):** 1 x 60 mL.  
   Blue colored. Ready to use. Contains: PBS Buffer, BSA, < 0.1 % NaN3.

5. **Wash Buffer, Concentrate (10x) (WASHBUF CONC):** 1 x 60 mL.  
   Contains: PBS Buffer, Tween 20.

6. **TMB Substrate Solution (TMB SUBS):** 1 x 15 mL.  
   Ready to use. Contains: TMB.

7. **TMB Stop Solution (TMB STOP):** 1 x 15 mL.  
   Ready to use. 0.5 M H2SO4.

8. **Adhesive Foil (FOIL):** 2 x.  
   For covering of Microtiter Plate during incubation.

9. **Plastic Bag (BAG):** 1 x.  
   Resealable. For dry storage of non-used strips.

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### Materials Required But Not Supplied

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 5; 50; 100; 500 µL
2. Calibrated measures
3. Tubes (1 mL) for sample dilution
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

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### Storage

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The unopened reagents are stable until the expiry date indicated. The Kit is stable up to 3 months after the first opening when the Microtiterplate is packed in a tightly closed bag, the bottles are closed with their screw caps and the kit is stored at 2-8°C.

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### Specimen Collection And Preparation

**Serum, Plasma (EDTA, Citrate):**  
The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Samples can store at 2-8°C for 2d, or at -20°C for longer time. Please keep away samples from heat or direct sun light. Avoid repeated freeze-thaw cycles.

### Dilution of Samples:

<table>
<thead>
<tr>
<th>Sample</th>
<th>to be diluted</th>
<th>with</th>
<th>Relation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma</td>
<td>generally</td>
<td>Diluent Buffer</td>
<td>1:101</td>
<td>e.g. 5 µL + 500 µL Diluent Buffer</td>
</tr>
</tbody>
</table>

Samples containing concentrations higher than the highest standard have to be diluted further.

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### Reagent Preparation
The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with 4 strips (32 determinations).

<table>
<thead>
<tr>
<th>Dilute / dissolve</th>
<th>Component</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mL</td>
<td>Wash Buffer</td>
<td>180 mL</td>
<td>bidist. water</td>
<td>1:10</td>
<td>Warm up at 37°C to dissolve crystals, if necessary. Mix vigorously.</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>

Assay Procedure

1. Pipette 100 µL of each Standard and diluted sample into the respective wells of the Microtiter Plate.
2. Cover plate with adhesive foil. Incubate 60 min at 18-25°C.
3. Remove adhesive foil. Discard incubation solution. Wash plate 3 x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
4. Pipette 100 µL of Enzyme Conjugate into each well.
5. Cover plate with new adhesive foil. Incubate 30 min at 18-25°C.
6. Remove adhesive foil. Discard incubation solution. Wash plate 3 x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
7. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8. Pipette 100 µL of TMB Substrate Solution into each well.
10. Stop the substrate reaction by adding 100 µL of TMB Stop Solution into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
11. Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 60 min after pipetting of the Stop Solution.

PROCEDURE NOTES:

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors (CV >10%).
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

Quality Control

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation
The evaluation of the test can be performed either qualitatively or quantitatively.

1. **Qualitative Evaluation:**
The Cut-off value is given by the optical density (OD) of the Standard B (Cut-off standard). The Cut-off index (COI) is calculated from the mean optical densities of the sample and Cut-off value. Samples with higher ODs are positive, samples with lower ODs are negative.

If the optical density of the sample is within a range of 20% around the Cut-off value (grey zone), the sample has to be considered as borderline. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

For a quantification, the Cut-off index (COI) of the samples can be formed as follows:

\[
\text{COI} = \frac{\text{OD Sample}}{\text{OD Standard B}}
\]

2. **Quantitative Evaluation:**
The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistic or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in Specimen Collection And Preparation and reassayed.

**Interpretation Of Results**

<table>
<thead>
<tr>
<th>Cytomegalovirus</th>
<th>n</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>IgG</td>
<td>176</td>
<td>43.8 %</td>
</tr>
<tr>
<td>IgM</td>
<td>88</td>
<td>1.1 %</td>
</tr>
</tbody>
</table>

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

**Typical Standard Curve**

Example. Do not use for calculation!

<table>
<thead>
<tr>
<th>Standard</th>
<th>U/mL</th>
<th>OD&lt;sub&gt;mean&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0.034</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>0.553</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>1.048</td>
</tr>
<tr>
<td>D</td>
<td>90</td>
<td>2.067</td>
</tr>
</tbody>
</table>
Reference Values

In an in-house study, apparently healthy subjects showed the following results:

<table>
<thead>
<tr>
<th>Method</th>
<th>Range</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative (Standard curve)</td>
<td>&lt; 8 U/mL</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>8 – 12 U/mL</td>
<td>equivocal</td>
</tr>
<tr>
<td></td>
<td>&gt; 12 U/mL</td>
<td>positive</td>
</tr>
<tr>
<td>Qualitative (Cut-off Index, COI)</td>
<td>&lt; 0.8</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.8 – 1.2</td>
<td>equivocal</td>
</tr>
<tr>
<td></td>
<td>&gt; 1.2</td>
<td>positive</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establishes its own range of normal values.

Performance Characteristics

<table>
<thead>
<tr>
<th>Analytical Specificity (Cross Reactivity)</th>
<th>No cross-reactivities were found to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Herpes 1, Rubella, Toxoplasma, Measles, Mumps, Varicella Parainfluenza and EBV-VCA Interferences of dsDNA IgG, TG IgG and TPO IgG positive samples or samples of donors suffering from an acute EBV infection cannot totally be excluded.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precision</th>
<th>Mean (U/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay</td>
<td>18.5 – 60.7</td>
<td>9.6 – 12.2</td>
</tr>
<tr>
<td>Inter-Assay</td>
<td>2.6 – 64.7</td>
<td>4.4 – 15.4</td>
</tr>
<tr>
<td>Inter-Lot</td>
<td>2.3 – 69.7</td>
<td>4.0 – 30.6</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>1.15 U/mL</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Linearity</th>
<th>Range (U/mL)</th>
<th>Serial dilution up to</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>58 – 227</td>
<td>1/4</td>
<td>70 – 113</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Mean recovery after spiking</th>
<th>87 – 100 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical specificity</td>
<td>98% (n = 98)</td>
<td></td>
</tr>
<tr>
<td>Clinical sensitivity</td>
<td>100% (n = 73)</td>
<td></td>
</tr>
</tbody>
</table>

Precautions

(1) For in-vitro diagnostic use only. For professional use only.
(2) Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
(3) In case of severe damage of the kit package please contact CD or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
(4) Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
(5) Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
(6) Reagents of this kit containing hazardous material may cause eye and skin irritations. See Reagents And Materials Provided and labels for details.
(7) Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
(8) Avoid contact with Stop solution. It may cause skin irritations and burns.
(9) Some reagents contain sodium azide (NaN3) as preservatives. In case of contact with eyes or skin, flush immediately with...
water. NaN₃ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.

(10) All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

**Limitations**

1. Specimen collection has a significant effect on the test results. See Specimen Collection And Preparation for details.
2. Azide and thimerosal at concentrations > 0.1% interfere in this assay and may lead to false results.
3. The following blood components do not have a significant effect (+/- 20% of expected) on the test results up to the below stated concentrations:
   (a) Hemoglobin: 8.0 mg/mL
   (b) Bilirubin: 0.3 mg/mL
   (c) Triglyceride: 5.0 mg/mL