ab108639

Cytomegalovirus IgG Human ELISA Kit

Instructions for Use

For the detection of Human Cytomegalovirus IgG concentrations in serum.

This product is for research use only and is not intended for *in vitro* diagnostic use.
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1. Introduction

ab108639, Cytomegalovirus (CMV) IgG ELISA Kit is intended for use in evaluating a patient's serologic status to CMV infection.

Cytomegalovirus is a herpes virus and a leading biological factor causing congenital abnormalities and complications among those who receive massive blood transfusions and immunosuppressive therapy. About half the pregnant women who contract a primary infection spread the disease to their fetus. When acquired in-utero, the infection may cause mental retardation, blindness, and/or deafness. Serological tests for detecting the presence of antibody to CMV can diagnose active or recent infection and provide valuable information regarding the history of previous infection. These tests are also useful in screening blood for transfusions in newborns and immuno-compromised recipients.
2. Assay Summary

Purified Cytomegalovirus (CMV) antigen is coated on the surface of microwells.

The test sample is added to the wells, and the CMV IgG specific-antibody, if present, binds to the antigen. All unbound materials are washed away.

HRP conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time.

The intensity of the color generated is proportional to the amount of CMV IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with standards and controls.
3. Kit Contents

- Microtiter Wells: purified CMV antigen-coated wells (12 x8 wells)
- Cytomegalovirus anti-IgG HRP Conjugate (red color): Red cap. 1 vial (12 ml)
- IgG Sample Diluent (green color): 1 bottle (22 ml)
- Cytomegalovirus IgG Standard 0 - 0 U/mL. Natural cap. (100 µl/vial)
- Cytomegalovirus IgG Standard 1 - 1.2 U/mL. Yellow cap. (100 µl/vial), CMV IgG index= 1.0
- Cytomegalovirus IgG Standard 2 - 6 U/mL. Red cap. (100 µl/vial)
- Cytomegalovirus IgG Standard 3 - 18 U/mL. Green cap. (100 µl/vial)
- Negative Control: Blue cap. (100 µl/vial)
- Positive Control: Purple cap. (100 µl/vial)
- 20X Washing Solution: 1 bottle (50 ml)
- TMB Substrate Solution, 1 vial (11 ml)
- Stop Solution: 1N HCl, Natural cap. 1 vial (11 ml)

4. Storage and Handling

Store the kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do
not exposure test reagents to heat, sun or strong light during storage or usage.

5. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

6. Preparation and Collection of Specimen

1. Collect blood specimens and separate the serum.

2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.
7. Assay Method

Assay Procedure:

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, Negative Control, Positive Control, and Standards by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. Dispense 100 µl of diluted sera, Standards, and Controls into the appropriate wells. For the reagent blank, dispense 100 µl of Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
6. Dispense 100 µl of Enzyme Conjugate into each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely. Note: Make sure there are no air bubbles in well before reading.

13. Read O.D. at 450 nm within 15 minutes with a microwell reader.

### 8. Data Analysis

1. Calculate the mean of duplicate Cytomegalovirus IgG Standard 1 value $x_c$.

2. Calculate the mean of duplicate positive control ($x_h$), negative control ($x_i$) and test samples ($x_s$).

3. Calculate the CMV IgG Index of each determination by dividing the mean values of each sample ($x$) by mean value of Cytomegalovirus IgG Standard 1, $x_c$.

**Typical Data**

**NOTE:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.
<table>
<thead>
<tr>
<th>CMV IgG (IU/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>1.2</td>
<td>0.930</td>
</tr>
<tr>
<td>6</td>
<td>1.496</td>
</tr>
<tr>
<td>12</td>
<td>2.167</td>
</tr>
</tbody>
</table>
9. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>Issue</td>
<td>Resolution</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation &amp; and optimal sample storage conditions (eg. minimize freeze/thaw cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
<td></td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “contact us” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).