（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Human Cytomegalo Virus (HCMV) IgG ELISA Kit

Catalog No: E-HD-E038

96T/96T*2

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2024.03.14

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the HCMV-IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified HCMV antigen. Samples are added to the ELISA Microtiter plate wells and the HCMV antibody in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugated Mouse-anti-human IgG antibody is added to each well and react with the compound to form "HCMV antigen- HCMV antibody-HRP antibody" compound. The TMB substrate is added to initiate the color developing reaction. The presence of HCMV-IgG can be determined according to the OD value after colorimetric assay with the Microplate reader.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
20×Concentrated Wash Buffer	50 mL
Sample Diluent	12 mL
HRP Conjugate	12 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Microplate reader with 450nm wavelength filter or dual-wavelength (450/630nm)

High-precision transferpettor, EP tubes and disposable dropper tips

37℃ Incubator or water bath

Deionized water

Absorbent paper

Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the Microplate Reader.
7. **Each reagent is optimized for use in the E-HD-E038. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E038 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and expiry date

Store unopened at 2 to 8℃. Do not freeze.

Please store the opened plate at 2 to 8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the box.

Sample preparation

1. **Serum:** Fresh collected serum should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated. Obviously contaminated samples can't be used.
2. Anticoagulant (EDTA, sodium citrate and heparin) in samples do not affect the result of the experiment in general. Endogenous interference substances in serum such as blood lipids, cholerythrin, hemoglobin, rheumatoid factors positive samples, AFP positive samples and pregnant samples will not affect the results normally. Common positive samples of specific virus antibodies, such as HAV, HBV, HCV, EB, HSV, RV and related diseases, will not affect the results.
3. Do not use heat inactivated samples, heat inactivation will degrade antibodies.
4. Samples can be stored at 2-8℃ for one week or stored at -20℃ for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
5. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control, 3 wells for negative control and 1 well for positive control. **Samples need test in duplicate** (Blank well is not necessary for dual-wavelength detection).
2. **Add sample:**
   a) Add 100 μL of **Positive/Negative Control** respectively to **Positive/Negative Control** wells, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection)
   b) Dilute the tested **Serum** with **Sample Diluent** at 1:10 (add 100 μL of Sample Diluent to the reaction well, and then add 10 μL of serum sample), mix fully.
3. **Incubate:** Gently tap the plate to ensure thorough mixing. Cover the ELISA plate with sealer. Incubate for 30 min at 37℃.
4. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60 sec each time.
5. **HRP conjugate:** Add 100 μL of **HRP Conjugate** to each well except the blank control well.
6. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37℃.
7. **Wash:** Repeat step 4.
8. **Add substrate:** Add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 10 min at 37℃ in shading light.
9. **Stop reaction:** Add 50 μL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. **OD Measurement:** Set the Microplate reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection. **This step should be finished in 30 min after stop reaction.**

Reference value

Normally, blank well (just chromogenic agent and stop solution): A450 ≤ 0.08. Positive control (PC): A450 > 0.30. Negative control (NC): A450 < 0.08.

Interpretation of test results

Calculate the Cut Off: Cut Off (C.0) = 0.10 + A value of average negative control (NC) (when A450 of average NC < 0.05, calculate at 0.05; while A450 of average NC ≥ 0.05, calculate at the actual value).

1. Positive result: A450 of Sample ≥ Cut Off.
2. Negative result: A450 of Sample < Cut Off.
3. Negative result indicates no HCMV-IgG antibody detected in samples, while positive result means the opposite.
4. The positive result of HCMV-IgG antibody is an important index of HCMV infection.

Limitations of test method

1. This test is only used as the qualitative detection of HCMV-IgG antibody in serum of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.