Technical Manual

Human Cytomegalovirus (HCMV) IgM ELISA Kit

- Catalogue Code: HDES0028
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Capture-ELISA as the method to detect human Cytomegalo virus IgM antibodies (HCMV-IgM) in serum or plasma samples. The ELISA Microtiter plate provided in this kit has been pre-coated with Mouse-anti-human IgM (μ chain). Samples are added to the ELISA Microtiter plate wells and the IgM antibody in which will be captured. Free components are washed away. Recombinant antigen HCMVrp52-65 conjugated with HRP is added to each well and react with the compound to form "Mouse-anti-human IgM (μ chain)-HCMV IgM - HCMVrp52-65 antigen-HRP" compound. The TMB substrate is added after washing to initiate the color developing reaction. The presence of HCMV-IgM can be determined according to the OD value after colorimetric assay with the Microplate reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
20×Concentrated Wash Buffer	50 mL
HRP Conjugate	12 mL
Sample Diluent	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

3. Other materials required but not supplied

- Microplate reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper

4. 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 HDES0028. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0028 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° C. Do not freeze.

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

Expiry date: expiration date is on the box.

5. Sample preparation

1. **Serum/plasma:** Human serum and plasma can be used as detected sample. Fresh collected samples should be fully centrifuged, and then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not precipitated fully. 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, but samples with hyperlipidemia, hemolysis or high protein may cause a false result.
3. Do not use heat inactivated samples, heat inactivation will degrade antibodies.
4. Samples can be stored at 2-8° C for one week or stored at -20° C 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.

6. Assay procedure

Restore all reagents and samples to room temperature (25° C) 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° C.

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/plasma** with **Sample Diluent** at 1:10 (add 100 µL of Sample Diluent to the reaction well, and then add 10 µL of serum/plasma 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° C.
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° C.
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 15 min at 37° C 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.**

7. 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.

8. 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.

9. Limitations of test method

1. This test is only used as the qualitative detection of HCMV-IgM antibody in serum and plasma 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.

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