Technical Manual

Human Mumps Virus (MuV) IgG ELISA Kit

- Catalogue Code: HDES0002
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect -ELISA as the method to detect the MuV-IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with MuV-IgG antigen. Samples are added to the ELISA Microtiter plate wells and the IgG antibody in which will be captured. Free components are washed away (including the specific IgG antibody). The “MuV antigen + anti-MuV-HRP conjugated antibody” is added to each well, the MuV-IgG antibody in captured IgG will specific bind to the MuV antigen + anti-MuV-HRP conjugated antibody. The TMB substrate is added after washing to initiate the color developing reaction. The presence of MuV-IgG can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

2. Kit components

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

3. Other materials required but not supplied

- Micro-plate 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
- Loading slot for Wash Buffer

4. Notes

1. 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.
2. 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.
3. 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.
4. Concentrated Wash Buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should be kept fresh.
7. The results shall depend on the readings of the Micro-plate Reader.
8. **Each reagent is optimized for use in the HDES0002. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0002 with different lot numbers.**

5. Storage and expiry date

Please store the opened kit at 2-8° C, protect from light and moisture. The shelf life of the opened kit is up to 1 months.

Expiry date: expiration date is on the box.

6. Requirements of sample

1. **Serum:** Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated.
2. Avoid of samples with hyperlipidemia (triglyceride > 20g/L), hemolysis (hemoglobin > 10 g/L) or jaundice (bilirubin > 0.2 g/L). Obviously contaminated samples can't be detected.
3. Samples can be stored at 2-8° C for one week. If samples not tested in a week, store them at -20° C and avoid freeze-thaw cycles.
4. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) for 30 min 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 2 well for positive control. **Samples need test in duplicate** (Blank well is not necessary for dual-wavelength detection).
2. **Add sample:**
   (1) 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)
   (2) 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° 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 Working Solution** 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 micro-plate 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 10 min after stop reaction.

8. Reference value

Normally, blank well (just chromogenic agent and stop solution) absorbance ≤ 0.08. Positive control (PC) A_{450} > 0.80. Negative control (NC) A_{450} < 0.10.

9. Interpretation of test results

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

value).

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

10. Limitations of test method

1. This test is only used as the qualitative detection of MuV -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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com