Technical Manual

Human Measles Virus IgM ELISA Kit

- **Catalogue Code: HDES0039**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This ELISA kit uses Capture-ELISA as the method to qualitatively detect the Measles Virus-IgM (MV-IgM) in human serum and plasma. 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 (including the specific IgG antibody). The MV antigen and anti-MV-antibody-HRP conjugated reagent are added to each well and incubate. The “anti-μ chain- MV-IgM antibody-MV antigen-HRP conjugated antibody” compound will form if the sample contains MV-IgM. Free components are washed away. The TMB substrate is added to initiate the color developing reaction and form a blue product. The presence of MV-IgM can be determined according to the OD value after colorimetric assay with the Micro-plate reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
MV Antigen	6 mL
Sample Diluent	12 mL
HRP Conjugate	6 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 bags	1 piece
Manual	1 copy

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

3. Other materials required but not supplied

- Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)
- 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 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. 20×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. Do not use components from different batches of kit.

5. Storage and expiry date

Store unopened at 2-8° C. Do not freeze.
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. Sample preparation

1. **Serum/plasma:** Human serum and plasma can be used as detected sample. Fresh collected serum specimens should be fully centrifugal, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated.
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 may not affect the results. Common positive samples of specific virus antibodies, such as HAV, HBV, HCV, EB, HSV, RV and related diseases, will not affect the results.
3. Samples can be stored at 2-8° C for one week. If samples not tested in a week, store them below -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, then dilute it with deionized water at 1:19.

7. 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 2 wells 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 **Negative/Positive Control** respectively to 3 negative control wells, 2 positive control wells, keep the blank control well empty.
   b) Dilute the tested Serum/plasma with **Sample Diluent** at 1:10 into sample well (add 100 µL of Sample Diluent and add 10 µL of **Serum/plasma** sample), mix fully.
3. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 60 min at 37° C in shading light.
4. **Wash:** 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. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **Add antigen and HRP conjugate:** Add 50 µL of **MV antigen** into each well except the blank control well and gently shake the plate to mix fully. Then add 50 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 20 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. **Note: Read the results within 30 min.**

8. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: $A_{450} \leq 0.08$; positive control (PC): $A_{450} \geq 0.60$ and average A value of negative control (NC): $A_{450} \leq 0.08$.

9. Interpretation of the results

Cut Off = 0.10 + average A value of negative control (NC) (when average A_{450} of NC ≤ 0.05, calculate at 0.05; while average A_{450} of NC > 0.05, calculate at the actual value).

1. Positive result: A_{450} of Sample ≥ Cut Off.
2. Negative result: A_{450} of Sample < Cut Off.
3. Negative result indicates that no MV-IgM antibody was detected in samples, while positive result means the opposite.

10. Limitations of test method

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

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