Technical Manual

Human Rubella Virus IgM ELISA Kit

- **Catalogue Code: HDES0023**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This ELISA kit uses Capture-ELISA as the method to detect the RV-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). HRP conjugated RV antigen is added to each well and react with the compound to form “Mouse-anti-human IgM (μ chain)-RV IgM - RV antigen-HRP” compound. The TMB substrate is added after washing to initiate the color developing reaction. The presence of RV-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
Sample Diluent	12 mL
HRP Conjugate	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. This kit is for research use only. It is disposable and cannot be used repeatedly.

2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. 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.
4. 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.
5. 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.
6. 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.
7. The results shall depend on the readings of the micro-plate Reader.
8. Do not use components from different batches of kit.
9. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Serum/plasma:** Human serum and plasma can be used as detected sample. Fresh collected specimens should be fully centrifugal, then take clear liquid for test, if not fully precipitation, suspended fibrous protein may cause a false positive. Obviously contaminated samples can't be used.
2. Anticoagulant (EDTA, sodium citrate and heparin) in samples do not affect the result of the experiment. Endogenous interference substances in samples such as blood lipids, cholerythrin, hemoglobin, and rheumatoid factors, HCV, Treponema Pallidum antibody positive samples may not affect the results in general.
3. Do not use heated inactivated samples. Heat inactivation will degrade antibodies.
4. Samples can be stored at 2-8° C for one week and 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, 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 1 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.
   (2) Dilute the tested **Serum/plasma** with **Sample Diluent** at 1:10 into sample well (add 1 00 μL of sample diluent and add 10 μL of sample), mix fully.
3. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA plate with sealer. Incubate for 30 min at 37° C in shading light.
4. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add **Wash buffer** to each well and immerse for 30 s each time. Repeat wash procedure for 5 times, 30 sec intervals/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. **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 in shading light.
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 mix thoroughly. Cover with a new plate sealer. Incubate for 15 min at 37° C in shading light (The reaction time can be extended according to the actual color change).
9. **Stop reaction:** Add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
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 30 min after stop reaction.**

8. 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.

9. 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 there is no RV-IgM antibody detected in samples, while positive result means the opposite.
4. The positive result of RV-IgM antibody is an important index of RV infection.

10. Limitations of test method

1. This test is only used as the qualitative detection of RV-IgM antibody in serum/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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