Technical Manual

Human Rubella Virus IgG ELISA Kit

- **Catalogue Code: HDES0024**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the RV-IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified RV antigen. Samples are added to the ELISA Microtiter plate wells and the RV 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 “RV antigen- RV antibody-HRP antibody” compound. The substrate reagent is added to initiate the color developing reaction. The presence of RV-IgG 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
HRP Conjugate	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

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 unopened at 2-8° C. Avoid freeze.
Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8° C.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Serum:** Use the conventional method to prepare serum. 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. 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 in general. Common positive samples of specific virus antibodies, such as HAV, HBV, HCV, EB, TP and related diseases, will not affect the results in general.
3. 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.
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 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 **negative/positive control** respectively to 3 negative control wells, 1 positive control well. Keep the blank control well empty.
   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 mix thoroughly. Cover the ELISA plate with sealer. Incubate for 30 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. **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. Cover the plate sealer and mix thoroughly. Incubate for 10 min at 37° C in shading light.
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 essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 30 min.**

8. Reference value

Normally, blank well (just substrate reagent and stop solution) absorbance: $A_{450} \leq 0.08$;
positive control (PC): $A_{450} > 0.30$ and average A value of negative control (NC): $A_{450} < 0.08$.

9. Interpretation of the results

Use each test result independently. Determine the result according to the Cut Off value.
Cut Off (C.0) = 0.10 + A value of average negative control (NC) (when A_{450} of average NC < 0.05, calculate at 0.05; while A_{450} of average 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 no RV-IgG antibody 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 RV-IgG 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.

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