Version 15-04/24

User's Manual

Rubella virus IgG ELSIA kit

REF **DEIA-NS2303-4**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This product is used for the in vitro qualitative detection of rubella virus IgG antibodies in human serum/plasma or whole blood samples.

General Description

Suitable for auxiliary diagnosis of rubella virus infection.

Rubella virus is an enveloped virus belonging to the Rubellaviridae family. It has only one serotype and has no cross-reactivity with other enveloped viruses. Rubella is an acute viral infection that generally affects only susceptible children and young adults worldwide. The occurrence of rubella is seasonal, with epidemics occurring every 5 to 9 years. The degree and periodicity of the epidemic are different in developed and developing countries, which may be related to the different immunization status of each country. Before the launch of large-scale rubella vaccination, the average age of children infected with rubella was 2 to 12 years old. Rubella virus spreads through the respiratory tract. It initially replicates in the nasopharyngeal mucosa and local lymph nodes. The incubation period is 12 to 23 days. The clinical symptoms of rubella are like those of a common cold. It usually begins with symptoms of upper flu and swollen lymph nodes behind the ears and suboccipital lymph nodes. Later, a latent red maculopapular rash appears on the face and quickly spreads throughout the body. Infection during pregnancy may cause fetal death or congenital rubella syndrome.

Antibodies can be detected 14 to 18 days after rubella virus infection, at which time maculopapular rash appears approximately simultaneously. Both lgM and lgG antibody titers initially increased, and then lgG continued to be at a higher titer, while lgM antibody titers decreased rapidly. The lgG level reached a peak 4 to 6 weeks after infection, and then gradually dropped to a certain level. Lasts a lifetime.

At present, the clinical and laboratory detection methods of rubella virus IgG mainly include indirect hemagglutination inhibition test, latex agglutination test, enzyme-linked immunosorbent assay, one-way radial hemolysis test and fluorescence inhibition test.

Principles of Testing

The kit adopts the principle of enzyme-linked immunoassay indirect method.

This kit uses inactivated and purified rubella virus antigen to coat the microplate. After the sample is added to the microwell, the anti-rubella virus antibody and the solid-phase rubella virus antigen in the microwell form an antigen-antibody immune complex. Unbound substances are removed by washing, and horseradish peroxidase-labeled mouse anti-human IgG monoclonal antibody is added. , causing it to react to form an antigen-antibody-enzyme-labeled secondary antibody complex, wash the enzyme conjugate that did not participate in the reaction, and add TMB substrate to develop color. The depth of the color is proportional to the content of rubella virus IgG antibody.

Reagents And Materials Provided

1. Pre-coated plate: 12T×8, inactivated purified rubella virus antigen
2. Mouse anti-human IgG monoclonal antibody enzyme label: 10mlx1 bottle, HRP enzyme-labeled

3. Mouse anti-human IgG monoclonal antibody, containing stabilizer and biological preservatives
4. Concentrated washing solution (20×): 30ml×1 bottle, 20 times concentrated phosphate buffer, containing NaCl, Tween-20
5. Sample diluent: 50ml×1 bottle, buffer, stabilizer, etc.
6. Negative control substance: 0.5ml×1 bottle, inactivated human rubella virus IgG antibody negative serum, stabilizer
7. Positive control substance: 0.5ml×1 bottle, inactivated human rubella virus IgG antibody positive serum, stabilizer
8. Substrate A: 5.0ml×1 bottle, the main component is carbamide peroxide
9. Substrate B: 5.0ml×1 bottle, the main component is TMB
10. Stop solution: 5.0ml×1 bottle, 0.5M H_2SO_4 solution
11. Ziplock bag: 1 serving
12. Parafilm: 3 sheets

Materials Required But Not Supplied

A microplate reader with a wavelength of 450nm or a fully automatic enzyme immunoassay analyzer.

Storage

1. Store refrigerated at 2-8°C and away from light. Freezing is prohibited. The validity period is 12 months.
2. Unused pre-coated boards should be immediately put into a ziplock bag with desiccant and sealed, and the storage time at 2-8°C should not exceed one week.
3. Any unused reagents of other components should be capped immediately and stored in an environment of 2-8°C. The storage time should not exceed one week.
4. Please refer to the product label for the production date and expiration date of the product.

Specimen Collection And Preparation

1. Human serum/plasma or whole blood can be used as assay samples. Anticoagulants (heparin, EDTA, sodium citrate) have no interference with sample test results.
2. Serum/plasma samples can be stable for one week when refrigerated at 2-8°C, but it is recommended to be used within 72 hours; whole blood samples can be used immediately; if long-term storage is required, serum/plasma samples can be directly frozen and stored at -20°C and can be stable for one year. , whole blood samples can be diluted with sample diluent, aspirate the supernatant, and can be stored frozen at -20°C for one year. It is recommended that frozen samples be frozen and thawed no more than 3 times.
3. Hemolyzed and lipemic samples should be avoided, and contaminated samples should not be used.

Assay Procedure

1. Sample preparation

a. First add 500μl of sample diluent into a 1.5ml centrifuge tube. serial number.

b. Collect 10 µl of whole blood or 5 µl of serum/plasma, add it to the corresponding centrifuge tube, and shake gently immediately to mix.

c. If it is a whole blood sample, place the centrifuge tube at room temperature (or a 4°C refrigerator) and wait until all the red blood cells have settled to the bottom of the well, then take the supernatant for testing; if it is a serum or plasma sample, mix it well before use.

2. Remove the kit and samples from the refrigerated environment, place them for 30 minutes, and allow them to equilibrate to room temperature; adjust the incubator or water bath to 37±1°C.
3. Prepare washing liquid: add 1 times the volume of concentrated washing liquid to 19 times the volume of distilled water, purified water or deionized water, and mix well.
4. Add samples: For each test, set up 1 well for positive control and 1 well for negative control, and add 100 µl of the corresponding control solution to each well; set up 1 well for blank control, and add 100 µl of sample diluent; add diluted samples (full) to each of the remaining wells. Take 100 µl of supernatant from blood samples. Attach a sealing film and incubate at 37°C for 60 minutes. Carefully remove the sealing film and pour out the liquid in the well. Add 300 µl of diluted washing solution to each well, shake slightly for 10 seconds, pour it out, and pat dry. Repeat 5 times. If possible, set the parameters according to the above washing conditions and wash with a plate washer.
5. Add anti-human IgG antibody enzyme label: 100 µl per well (do not add to blank wells). Attach the sealing film and incubate at 37°C for 30 minutes. Carefully remove the sealing film and pour out the liquid in the well. Add 300 µl of diluted washing solution to each well, shake slightly for 10 seconds, pour it out, and pat dry. Repeat 5 times.
6. Color development: Add 50 µl each of substrate solution A and substrate solution B to each well, and mix well. Attach a sealing film and place at 37°C to develop color in the dark for 15 minutes.
7. Determination: Carefully remove the sealing film, add 50 µl of stop solution to each well, and mix well. Within 30 minutes, use a microplate reader with dual wavelength 450nm/630nm to measure the OD value of each well (when using a single wavelength of 450nm, you must use a blank well to zero).

Calculation

1. Negative control OD450 <0.3 and positive control OD450 ≥0.6, the test is established.
2. Cut-Off value = negative control OD450 value × 2.1, S/CO value = $OD450nm_{sample}$/Cut-Off

   0.9≤S/CO <1.1, suspicious, it is recommended to repeat the test;

   S/CO value ≥1.1, positive;

   S/CO value <0.9, negative

Interpretation Of Results

1. Positive result: indicates previous infection with rubella virus or production of rubella virus antibodies.
2. Negative result: indicates no previous infection with rubella virus or no antibodies to rubella virus.
3. Suspicious results: It is recommended to repeat the test or test again after two weeks to dynamically observe changes in antibody levels.

Specificity

This product is not compatible with measles virus IgG antibody-positive samples, mumps virus IgG antibody-positive samples, hepatitis A virus IgG-positive samples, hepatitis B virus surface antibody-positive samples, varicella-zoster virus IgG antibody-positive samples, and hepatitis C virus IgG antibody positive samples, Treponema pallidum IgG antibody positive samples, Human immunodeficiency virus IgG antibody positive samples, Epstein-Barr virus IgG antibody positive samples, Mycoplasma pneumoniae IgG antibody positive samples, Cytomegalovirus IgG antibody positive samples, Herpes simplex virus IgG antibody positive samples , Toxoplasma IgG antibody-positive samples cross-reacted. This product has not been studied on non-specific high IgG, non-specific high IgM, and HAMA samples, so clinical use should be avoided.

Interferences

Clinically, hemolyzed samples and lipemia samples may affect experimental results and should be avoided; samples containing autoantibodies (rheumatoid factor, antinuclear antibodies) and pregnant woman serum samples will not interfere with this product.

Precautions

1. This product is only for in vitro diagnosis, one-time use, and cannot be reused.
2. Please strictly follow the instructions. Unauthorized changes may cause unreliable results.
3. The same components in our company's kits with different batch numbers and different varieties must not be mixed. Do not mix with reagents from other manufacturers. Do not use reagents that have expired.
4. Required utensils and consumables not provided in the kit: distilled water, purified water or deionized water; 10μl, 200μl, 1000μl pipettes; pipettes; incubator or water bath; containers and measuring tools, etc.
5. Samples should be taken accurately when diluting. When adding samples, a micro-injector should be used to add samples accurately.
6. Do not use tap water to dilute the washing solution or wash the plate.
7. The sealing film can only be used once to avoid cross-contamination.
8. The interval between adding substrate solution A and substrate solution B should not exceed 2 minutes. The light blue substrate solution B visible to the naked eye should be discarded.
9. The stop solution contains H_2SO_4, please pay attention to safety when using it.
10. All samples and waste after testing should be considered infectious and disposed of in accordance with relevant biosafety regulations.

Limitations

It is only used for the qualitative detection of Rubella virus IgG antibodies in humans.