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User's Manual

Mumps virus IgG Antibody ELSIA Kit

REF **DEIA-NS2303-8**

Σ **96T**

RUO

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 qualitative in vitro detection of mumps virus IgG antibodies in human serum/plasma or whole blood samples.

Suitable for auxiliary diagnosis of mumps virus infection; mumps virus IgG antibody detection in vivo after vaccination with mumps component vaccine and mumps virus IgG antibody detection in normal or susceptible people.

General Description

Mumps virus belongs to the genus Paramyxovirus in the family Paramyxoviridae and has only one serotype. It can be divided into different genotypes based on sequence differences in genes encoding small hydrophobin proteins (SH genes). The virus is a single-stranded RNA virus, surrounded by a lipid membrane from the host cell. There are two components on the virus surface, hemagglutinin-neuraminidase protein and lytic protein, which play an important role in the virulence of the virus. Antibodies to the hemagglutinin-neuraminidase protein neutralize the virus, which can replicate in many cells, including chicken embryo cells.

The initial symptoms of mumps are generally non-specific, such as myalgia, headache, malaise, and low-grade fever. Typical unilateral or bilateral parotid gland swelling will appear within 1 day. In 10% of cases, symptoms of obvious involvement of other salivary glands can be seen. After about a week, the fever and swelling of the parotid gland subside, and unless complications occur, the disease usually resolves. About 30% of cases have only non-specific symptoms or no symptoms. Most infections in children under 2 years old are subclinical. They are contagious from 2 days before parotid gland enlargement to 9 days after parotid gland enlargement. There is no specific treatment. Although complications may occur, mumps is generally a mild, self-limiting disease with a mortality rate of only 1/10,000. 50% to 60% of patients may develop cerebrospinal fluid leukocytosis (>5/mm3), but no encephalitis/ Meningitis manifestations; up to 15% of patients have symptoms of meningitis. Mumps encephalitis occurs in 0.02% to 0.3% of cases. Although the mortality rate of mumps encephalitis is low, permanent sequelae can occur, such as paralysis, convulsions, cranial nerve paralysis, aqueductal stenosis, and cerebral edema. Acquired sensorineural deafness caused by mumps is one of the main causes of childhood deafness, occurring in approximately 5/100,000 mumps patients. About 20% of post-pubertal males suffering from mumps will develop orchitis, of which 20% may involve both sides. However, orchitis secondary to mumps rarely causes infertility. Symptomatic oophoritis and mastitis are rare and do not have permanent sequelae. Spontaneous miscarriage may occur in 25% of cases of mumps infection within 12 weeks of pregnancy. However, there have been no reports of fetal malformation if mumps virus infection occurs during pregnancy. Pancreatitis occurs in approximately 4% of cases, but the relationship between mumps-induced pancreatitis and diabetes is uncertain.

Life-long protection is generally obtained after natural infection, but recurrence of mumps has also been reported. IgA antibodies secreted by the nasopharyngeal mucosa have the effect of neutralizing the mumps virus and can be regarded as the first line of defense. Immunity to mumps is associated with serum-specific antibodies. It is unclear whether lifelong immunity must be achieved through natural boosting of the body's immune system by wild viruses circulating in the human population. Detection of serum-specific IgG antibodies using commonly used immunological methods can demonstrate the body's immunity.

At present, the main methods for detecting mumps virus-specific IgG antibodies include: neutralization test, hemagglutination inhibition test, enzyme-linked immunoassay (ELISA), etc.

Principles of Testing

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

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

Reagents And Materials Provided

1. Pre-coated plate: 12T×8, inactivated purified mumps virus antigen

2. Mouse anti-human IgG monoclonal antibody enzyme label: 10ml × 1 bottle, HRP enzyme-labeled mouse anti-human IgG monoclonal antibody, containing stabilizer and biological preservatives

3. Concentrated washing solution (20×): 30ml×1 bottle, 20 times concentrated phosphate buffer, containing NaCl, Tween-20

4. Sample diluent: 50ml×1 bottle, sodium citrate buffer, stabilizer, etc.

5. Negative control substance: 0.5ml×1 bottle, inactivated human anti-mumps virus IgG negative serum, stabilizer

6. Positive control substance: 0.5ml×1 bottle, inactivated human anti-mumps virus IgG positive serum, stabilizer

7. Substrate A: 5.0ml×1 bottle, the main component is carbamide peroxide

8. Substrate B: 5.0ml×1 bottle, the main component is TMB

9. Stop solution: 5.0ml×1 bottle, 0.5M H_2SO_4 solution

10. Ziplock bag: 1 serving

11. 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 should not exceed one week in an environment of 2-8°C.
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 measurement samples. Anticoagulants (heparin, EDTA, sodium citrate) have no interference with sample test results.
2. Serum/plasma samples can be stable for 4 days 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 at -20°C. It is stable for one year. The whole blood sample can be diluted with sample diluent and the supernatant is aspirated out. It can be stable for one year when frozen and stored at -20°C. It is recommended that frozen samples be frozen and thawed no more than 3 times.
3. Avoid using hemolyzed and lipemic samples, and do not use contaminated samples.

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. Take the supernatant for testing; if it is a serum or plasma sample, mix it well before use.

2. Take out the kit and samples from the refrigerated environment, place them for 30 minutes, and balance to room temperature; adjust the incubator or water bath to 37±1°C.
3. Prepare washing solution: 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, 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: S/CO value ≥ 1.1, indicating that the mumps virus IgG antibody in the sample is positive.
2. Negative result: S/CO value <0.9, indicating that the mumps virus IgG antibody in the sample is negative.
3. Suspicious results: 0.9 ≤ S/CO value < 1.1, indicating that the mumps virus IgG antibody in the sample is at a critical value. It is recommended to repeat the test or test again after two weeks to dynamically observe changes in antibody levels.

Precision

CV% < 15%

Specificity

This product is not compatible with rubella virus IgG antibody positive samples, measles 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, 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, Cross-reactivity occurred in Toxoplasma IgG antibody-positive samples. 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

Hemolysis with a hemoglobin concentration of 2.0 mg/ml, lipemia with a triglyceride concentration of 6 mmol/L, and jaundice with a bilirubin concentration of 100 μmol/L have no effect on the test results. Samples containing autoantibodies (rheumatoid factor, antinuclear antibodies) and serum samples from pregnant women 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 auxiliary diagnosis of mumps virus infection and the qualitative detection of mumps virus IgG antibodies.