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User's Manual

Diphtheria toxoid IgG ELISA kit

REF **DEIA-NS2303-2**

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 diphtheria toxoid IgG antibodies in human serum/plasma or whole blood samples.

General Description

It is suitable for auxiliary diagnosis of diphtheria bacilli infection, detection of whether the level of anti-diphtheria toxoid IgG antibodies in normal or susceptible people reaches the full protective titer (0.1IU/ml), and detection of anti-diphtheria toxoid IgG antibody levels after vaccination with vaccines containing diphtheria toxoid components. Whether the immune success standard (0.1IU/ml) is reached.

Diphtheria is an acute upper respiratory tract infection caused by Gram-positive Corynebacterium diphtheriae. Bacterial toxins can cause the production of upper respiratory tract obstructive pseudomembranes or damage myocardium and other tissues, leading to illness and death. Historically, many countries have experienced diphtheria epidemics that mainly affected children. In countries where diphtheria is endemic, the disease mostly occurs as sporadic cases or small outbreaks. Although most B. diphtheriae infections are asymptomatic or clinically relatively mild, case fatality rates are high (>10%), even in recent outbreaks.

Diphtheria bacilli are elongated, rod-shaped, Gram-positive bacilli with 4 biotypes (severe, belfanti, mild, and intermediate). In addition to bacterial exotoxins, cell wall components such as O antigen and K antigen play an important role in the pathogenicity of diphtheria. The heat-stable O antigen is the same for all Corynebacterium species, while the heat-labile K antigen varies from strain to strain and can be used to identify strains. At the same time, K antigen is important for mucosal adhesion, and invasiveness is mediated by cord factors (toxic glycolipids). The most important virulence factor of B. diphtheriae is exotoxins, which are highly conserved polypeptides mediated by bacteriophages and encoded by bacterial chromosomes. The exotoxin has very low activity outside the host cell, but after adhesion and entry into the cell through the non-toxic fragment B, the toxic fragment A dissociates and kills the cell by inhibiting cellular protein synthesis. Diphtheria exotoxins can cause local and systemic cell death.

In most cases, infection in diphtheria-susceptible individuals only results in transient pharyngeal bacterial carriage without illness. Contamination from skin wounds can cause cutaneous diphtheria and, occasionally, mucosal infections outside the respiratory tract. Skin and mucosal lesions are important sources of infection and occasionally cause systemic pathological reactions. Symptoms of respiratory diphtheria generally appear after an incubation period of 1 to 5 days. The onset of the disease is slow and is characterized by moderate fever and mild exudative pharyngitis. In severe cases, so-called pseudomembranes gradually form in the larynx, which have a typical asymmetrical gray-white appearance and are tightly adherent to deeper tissues. Pseudomembranes can extend into the nasal cavity and larynx, causing airway obstruction. Laryngeal diphtheria sometimes occurs without pharyngeal symptoms and is an emergency that often requires tracheotomy. Exotoxins absorbed through mucosal (or skin) lesions can cause toxic damage to the myocardium, kidneys, nervous system and other organs.

Immunity against severe local disease and systemic disease relies primarily on the antitoxin antibody IgG, whereas type-specific protection against vector and mild local disease is the induction of antibodies against cell wall variant K antigens. Cellular immunity may also play a protective role. However, sometimes infection

does not induce protective immunity. There is no protection at circulating antitoxin levels below 0.01 IU/ml, partial protection at 0.01 IU/ml, complete protection at ≥0.1 IU/ml, and antitoxin levels of 1.0 IU/ml are associated with long-term protective immunity. The antitoxin crosses the placenta and the newborn acquires passive immunity during the first few months of life.

At present, the main methods for detecting diphtheria toxoid-specific IgG antibodies include: Schick test, cell neutralization test, enzyme-linked immunoassay (ELISA), etc.

Principles of Testing

The kit is an indirect enzyme-linked immunosorbent assay.

This kit uses refined diphtheria toxoid antigen to coat the microplate. After the sample is added to the micropore, the anti-diphtheria toxoid antibody and the solid-phase diphtheria toxoid antigen in the micropore form an antigen-antibody immune complex. 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 diphtheria toxoid IgG antibody.

Reagents And Materials Provided

1. Pre-coated plate: 12T×8, diphtheria toxoid 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-diphtheria toxoid IgG negative serum, stabilizer
6. Positive control substance: 0.5ml×1 bottle, inactivated human anti-diphtheria toxoid 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 at 2-8°C should not exceed one week.
3. Any unused reagents of other components should be capped immediately and stored at 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 the 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 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

The cut-off value of this kit is set at a fully protective titer of diphtheria toxoid IgG antibody of 0.1IU/ml (original sample).

1. Positive result: It indicates that the diphtheria bacillus has been infected in the past or has been vaccinated with a vaccine containing diphtheria toxoid components and has produced antibodies. The antibody level is not less than 0.1IU/ml, reaching a complete protective titer.
2. Negative result: It indicates that the level of diphtheria toxoid IgG antibody in the body is lower than 0.1IU/ml, which means the full protective titer has not been reached.
3. Invalid results: For suspicious results, it is recommended to repeat the test or test again after two weeks, and dynamically observe changes in antibody levels.

Performance Characteristics

1. Compliance rate of positive reference products: 10 positive reference products from the company were tested, and the results were all positive.
2. Compliance rate of negative reference products: 10 corporate negative reference products were tested, and the results were all negative.

Precision

CV% < 15%

Specificity

This product is not compatible with pertussis toxoid and filamentous hemagglutinin IgG antibody-positive samples, tetanus toxoid 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, and Toxoplasma gondii IgG antibody-positive samples had cross-reactions. 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 qualitative detection of diphtheria toxoid IgG antibodies in humans.