Version 02-09/24

User's Manual

Clostridium Perfringens NetB ELISA Kit

REF **DEIASL175W**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit is used to determine the content of NetB toxin (NetB) of Clostridium perfringens in microbial samples.

Principles of Testing

This kit uses the double antibody sandwich method to determine the level of NetB toxin (NetB) of Clostridium perfringens in the sample. The purified NetB toxin (NetB) antibody of Clostridium perfringens is coated on the microwell plate to prepare a solid phase antibody. NetB toxin (NetB) of Clostridium perfringens is added to the microwells coated with the monoclonal antibody in sequence, and then combined with the HRP-labeled NetB toxin (NetB) antibody of Clostridium perfringens to form an antibody-antigen-enzyme-labeled antibody complex. After thorough washing, the substrate TMB is added for color development. TMB is converted into blue under the catalysis of HRP enzyme, and converted into the final yellow under the action of acid. The depth of color is positively correlated with the NetB toxin (NetB) of Clostridium perfringens in the sample. The absorbance (OD value) is measured at a wavelength of 450nm using an enzyme reader,

Reagents And Materials Provided

1. 30× Wash Buffer, 20ml
2. Enzyme Conjugate, 6ml
3. Coating Plate, 12×8
4. Sample Diluent, 6ml
5. Substrate A, 6ml
6. Substrate B, 6ml
7. Stop Solution, 6ml
8. Standard 120pg/ml, 0.5ml
9. Standard Diluent, 1.5ml

Materials Required But Not Supplied

1. ELISA reader
2. Pipette and tip
3. Plate washer
4. Test tubes, centrifuge tubes, measuring cylinders, etc.
5. Distilled water or deionized water
6. Electric thermostat

Storage

1. Storage: 2-8°C

2. Validity period: 6 months

Specimen Collection And Preparation

Collection of liquid samples

1. Serum: Collect in sterile tubes, let blood coagulate naturally at room temperature for 10-20 minutes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), and carefully collect the supernatant. If precipitation occurs during storage, centrifuge again.
2. Plasma: EDTA, sodium heparin or sodium citrate should be selected as anticoagulant according to the requirements of the specimen. After mixing for 10-20 minutes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), and carefully collect the supernatant. If precipitation forms during storage, centrifuge again.
3. Urine: Collect in sterile tubes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. If precipitation forms during storage, centrifuge again.
4. Pleural and abdominal fluid: Collect with sterile tubes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a precipitate is formed during storage, centrifuge again.
5. Cerebrospinal fluid: Collect with sterile tubes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a precipitate is formed during storage, centrifuge again.
6. Saliva: Collect with sterile tubes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a precipitate is formed during storage, centrifuge again.
7. Cell culture supernatant: When detecting secretory components, collect with sterile tubes. Centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a precipitate is formed during storage, centrifuge again.
8. Milk: Collect with sterile tubes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a precipitate is formed during storage, centrifuge again.
9. Honey: Collect with sterile tubes, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a precipitate is formed during storage, centrifuge again.
10. Whole blood: Collect with sterile tubes containing anticoagulants, shake gently immediately, and gently invert back and forth several times to mix the blood and anticoagulant to prevent blood coagulation.

Collection of solid samples

1. Tissue specimens: After cutting the specimen, weigh 1g of tissue and add 9ml of PBS at pH 7.2-7.4. Use a hand or homogenizer to homogenize the specimen thoroughly. Centrifuge for about 20 minutes (2000-3000 rpm) and carefully collect the supernatant. Aliquot one portion for testing and freeze the rest for later use. If precipitation forms during storage, centrifuge again. For plant tissues, if it is difficult to homogenize, grind it thoroughly in liquid nitrogen.
2. Intracellular protein samples: Many proteins to be tested are not secreted proteins. To detect intracellular proteins, it is necessary to collect cells first, wash them, and then use ultrasound to disrupt the cells and centrifuge to obtain the supernatant.

   (1) Cultured cells

   A. Animal cells: Dilute the cell suspension with PBS at pH7.2-7.4 to a cell concentration of about 1 million/ml. Use ultrasound to disrupt the cells to destroy them and release their intracellular components. Centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a

precipitate is formed during storage, centrifuge again.

B. Plant cells: Dilute the cell suspension with PBS at pH 7.2-7.4 to a cell concentration of about 1 million/ml, place on an ice box, and use an ultrasonic disruptor to set the mode of disrupting for 2s and cooling for 30s to fully disrupt the cells, so that the cells are destroyed and the intracellular components are released. Centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and if a precipitate is formed during storage, centrifuge again.

(2) Tissue cells

After cutting the specimen, weigh 1g of tissue, add 9ml of PBS at pH 7.2-7.4, and homogenize the specimen by hand or with a homogenizer. Centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), remove the supernatant, and then carefully wash the precipitated cells three times with PBS at about pH7.2-7.4. Use the above cell disruption method to disrupt the cells.

3. Throat swab: Add 2ml of PBS at about pH7.2-7.4, dissolve the head of the throat swab, shake well, take out the throat swab with tweezers and squeeze out the liquid, centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), and carefully collect the supernatant. Aliquot one portion for testing, and freeze the rest for later use. If a precipitate is formed during storage, centrifuge again. If you are testing secreted protein, take the supernatant directly for testing. If you are testing intracellular protein, you need to disrupt the cells.
4. Plant specimens:

   A. While maintaining the fresh weight, the weight of each sample should not be less than 50mg;

   B. The tissue homogenate ratio is 10%, that is, 1g tissue is added with 9mL homogenate, and the homogenate is PBS with a concentration of 0.01mol/l and a pH value of 7.2-7.4. If the sample weight is adjusted, the corresponding homogenate can be adjusted according to a ratio of 1:9;

   C. Cut the leaf tissue into pieces and put them into a mortar, grind them into powder with liquid nitrogen, and add the converted amount of homogenate;

   D. Centrifuge and take the supernatant, 4000-5000 rpm, time for 15 minutes.

Sample requirements

1. Extract the specimen as soon as possible after collection. The extraction should be carried out according to relevant literature. The experiment should be carried out as soon as possible after extraction. If the experiment cannot be carried out immediately, the specimen can be stored at -20°C, but repeated freezing and thawing should be avoided.
2. Samples containing NaN3 cannot be tested because NaN3 inhibits the activity of horseradish peroxidase (HRP).
3. The above are general sample processing methods and cannot cover all kinds of samples. For some special samples, it is recommended that the experimenter refer to the published literature and design a reasonable sample processing method by himself.

Assay Procedure

1. Dilution of standard: This kit provides one original standard. Users can dilute it in a small test tube according to the following.

   60pg/ml, Standard 5, 150μl of original standard added to 150μl of standard diluent

   30pg/ml, Standard 4, 150μl of Standard 5 added to 150μl of standard diluent

15pg/ml, Standard 3, 150μl of Standard 4 added to 150μl of standard diluent

7.5pg/ml, Standard 2, 150μl of Standard 3 added to 150μl of standard diluent

3.75pg/ml, Standard 1, 150μl of Standard 2 added to 150μl of standard diluent

2. Sample addition: Set up blank wells (blank control wells do not add samples and Enzyme Conjugate, and the rest of the steps are the same), standard wells, and sample wells to be tested. Accurately add 50μl of the standard sample to the coated plate, add 40μL of sample diluent to the sample well to be tested, and then add 10μl of the sample to be tested (the final dilution of the sample is 5 times). Add the sample to the bottom of the well of the enzyme-labeled plate, try not to touch the well wall, and gently shake to mix.
3. Incubation: Seal the plate with a sealing film and incubate at 37°C for 30 minutes.
4. Liquid preparation: Dilute the 30-fold concentrated washing solution with 30-fold distilled water for use.
5. Washing: Carefully peel off the sealing film, discard the liquid, shake dry, fill each well with washing solution, let it stand for 30 seconds and discard it, repeat this 5 times, and pat dry.
6. Add enzyme: Add 50μl of enzyme-labeled reagent to each well, except for the blank well.
7. Incubation: Same as 3.
8. Washing: Same as 5.
9. Color development: First add 50μl of color Substrate A to each well, then add 50μl of color Substrate B, gently shake and mix, and color development at 37°C in the dark for 10 minutes.
10. Stop: Add 50μl of stop solution to each well to stop the reaction (the blue color turns yellow immediately).
11. Determination: Zero the blank well and measure the absorbance (OD value) of each well in sequence at a wavelength of 450nm. The determination should be performed within 15 minutes after adding the stop solution.

Calculation

Draw a standard curve on the coordinate paper with the concentration of the standard as the horizontal axis and the OD value as the vertical axis. Find the corresponding concentration from the standard curve according to the OD value of the sample; then multiply it by the dilution factor; or use the concentration of the standard and the OD value to calculate the linear regression equation of the standard curve, substitute the OD value of the sample into the equation, calculate the sample concentration, and then multiply it by the dilution factor to get the actual concentration of the sample.

Performance Characteristics

1. The correlation coefficient R value between the sample linear regression and the expected concentration is above 0.95
2. The intra-batch coefficient of variation and the inter-batch coefficient of variation should be less than 10% and 12% respectively

Detection Range

1.6pg/ml- 70pg/ml