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User's Manual

Clostridium Perfringens NetB ELISA Kit

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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.

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 intended for detection of Clostridium perfringens Necrotic Enteritis Toxin B (NetB) concentration in chicken serum, plasma and related liquid samples.

General Description

Clostridium perfringens is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium of the genus Clostridium. C. perfringens is ubiquitous in nature and can be found as a normal component of decaying vegetation, marine sediment, the intestinal tract of humans and other vertebrates, insects, and soil.C. perfringens is commonly encountered in infections as a benign component of the normal flora. In this case, its role in disease is minor. Infections due to C.perfringens show evidence of tissue necrosis, bacteremia, emphysematous cholecystitis, and gas gangrene, which is also known as clostridial myonecrosis.

Principles of Testing

This kit uses the double antibody sandwich method to determine the level of Clostridium perfringens Necrotic Enteritis B (NetB) in the specimen. Pre-coated the microplate with purified Clostridium perfringens necrotizing enteritis B (NetB) antibody to make a solid phase antibody, and then add standard or sample to the microwell in turn, the NetB in the sample will be combined with HRP The labeled NetB antibody binds 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 into the final yellow under the action of acid. The color intensity is positively correlated with the NetB in the sample. The absorbance (OD value) was measured with a microplate reader at a wavelength of 450nm, and the concentration of Clostridium perfringens necrotizing enteritis B (NetB) in the sample was calculated from the standard curve.

Reagents And Materials Provided

1. Microplate precoated with NetB polyclonal antibody: 12x8 strips
2. HRP-Conjugate Solution: 6ml
3. 30x Wash Buffer: 20ml
4. Sample Diluent: 6ml
5. Substrate A: 6ml
6. Substrate B: 6ml
7. Stop Solution: 6ml
8. Standard (240ng/L): 0.5ml
9. Standard Diluent: 1.5ml
10. Instruction
11. Cover Film

12. Sealed bag

Storage

This kit should be stored at a temperature of 2-8 ° C. The shlef life is 6 months.

Specimen Collection And Preparation

Serum: The blood is naturally coagulated at room temperature for 10-20 minutes, and then centrifuged for about 20 minutes (2000-3000 rpm). Collect the supernatant carefully. If precipitation occurs during storage, centrifuge again.

Plasma: EDTA or sodium citrate should be selected as the anticoagulant according to the requirements of the specimen. After mixing for 10-20 minutes, centrifuge for about 20 minutes (2000-3000 rpm). Collect the supernatant carefully. If a precipitate forms during storage, it should be centrifuged again.

Note:

1. The specimen should be extracted as soon as possible after collection, and the experiment should be carried out as soon as possible after extraction. If the test cannot be performed immediately, the specimen can be stored at -20°C, but repeated freezing and thawing should be avoided.
2. The kit cannot detect samples containing NaN3 since NaN3 will inhibit horseradish peroxidase (HRP) activity.

Reagent Preparation

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 580 mL of deionized or distilled water to prepare

600 mL of Wash Buffer.

NetB Standard - Label 5 standard tubes as Standard 1-5. Pipette 150μL of Standard Diluent into each standard tube. Pipette 150μL of 240ng/L Standard Solution into the Standard 5 and mix thoroughly to prepare the 120ng/L Standard Solution. Then prepare the series standard solution as the table below.

Standard	Concentration	Preparation with Standard Diluent
Standard 5	120ng/L	150μL Standard Solution (240ng/L) + 150μL Standard Diluent
Standard 4	60ng/L	150μL Standard 5 (120ng/L) + 150μL Standard Diluent
Standard 3	30ng/L	150μL Standard 4 (60ng/L) + 150μL Standard Diluent
Standard 2	15ng/L	150μL Standard 3 (30ng/L) + 150μL Standard Diluent
Standard 1	7.5ng/L	150μL Standard 2 (150ng/L) + 150μL Standard Diluent

Assay Procedure

1. Adding samples: Set up blank wells (the blank control wells do not add samples and enzyme-labeled reagents, the other steps are the same), standard wells, and sample wells to be tested. Accurately add 50μL of the standard sample on the enzyme-labeled coating plate, add 40μL of sample diluent to the test sample well, and then add 10μL of the test sample (the final dilution of the sample is 5 times). Add the

sample to the bottom of the well of the microtiter plate, try not to touch the wall of the well, shake gently to mix the solution.

2. Incubation: Seal the plate with a sealing film and incubate at 37°C for 30 minutes.
3. Washing: Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add enzyme: add 50μL of HRP-Conjugate Solution to each well, except for blank wells.
5. Incubation: Seal the plate with a sealing film and incubate at 37°C for 30 minutes.
6. Repeat the aspiration/wash as in step 3.
7. Color development: add 50μL of Substrate A and 50μl of Substrate B to each well, shake gently to mix, and develop color at 37°C for 10 minutes in the dark.
8. Stop Reaction: add 50μL stop solution to each well to stop the reaction (the blue turns to yellow).
9. Determination: Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

Calculation

Take the concentration of the standard substance as the abscissa and the OD value as the ordinate, draw a standard curve on graph paper, and find the corresponding concentration from the standard curve according to the OD value of the sample; then multiply by the dilution factor; or use the concentration of the standard substance Calculate the linear regression equation of the standard curve with the OD value. Substitute the OD value of the sample into the equation to calculate the sample concentration and multiply it by the dilution factor to obtain the actual concentration of the sample.

Detection Range

3ng/L-140ng/L

Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
3. The Stop Solution provided with this kit is an acid solution.
4. Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
5. Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
6. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on

our website prior to use.