Creative Diagnostics®

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**User's Manual**

# Human Clostridium Perfringens NetB ELISA Kit

REF **DEIASL178**

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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 kit is intended for detection of Clostridium perfringens Necrotic Enteritis Toxin B (NetB) concentration in human 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

In order to measure the concentration of nETB in the sample, this nETB ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus nETB concentration. The concentration of nETB in the samples is then determined by comparing the O.D. of the samples to the standard curve. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer.

## Reagents And Materials Provided

1. Microelisa stripplate: 12*8strips
2. Standard (S0 → S5): 0.3ml*6tubes. The concentration was followed by 0,1.5,3,6,12,24 ng/ml.
3. Sample Diluent: 6.0ml
4. HRP-Conjugate reagent: 10.0ml
5. 20X Wash solution: 25ml
6. Chromogen Solution A: 6.0ml
7. Chromogen Solution B: 6.0ml
8. Stop Solution: 6.0ml
9. Closure plate membrane: 2
10. User manual: 1
11. Sealed bags: 1

## Materials Required But Not Supplied

1. Standard microplate reader(450nm)

2. Precision pipettes and Disposable pipette tips.
3. 37°C incubator

## Storage

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

## Specimen Collection And Preparation

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C . Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000×g at 2-8°C within 30 minutes of collection. Store samples at -20°C or -80°C . Avoid repeated freeze-thaw cycles.

Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C . Avoid repeated freeze-thaw cycles.

Note: The samples shoule be centrifugated dequately and no hemolysis or granule was allowed.

## Reagent Preparation

20×wash solution: Dilute with Distilled or deionized water 1:20.

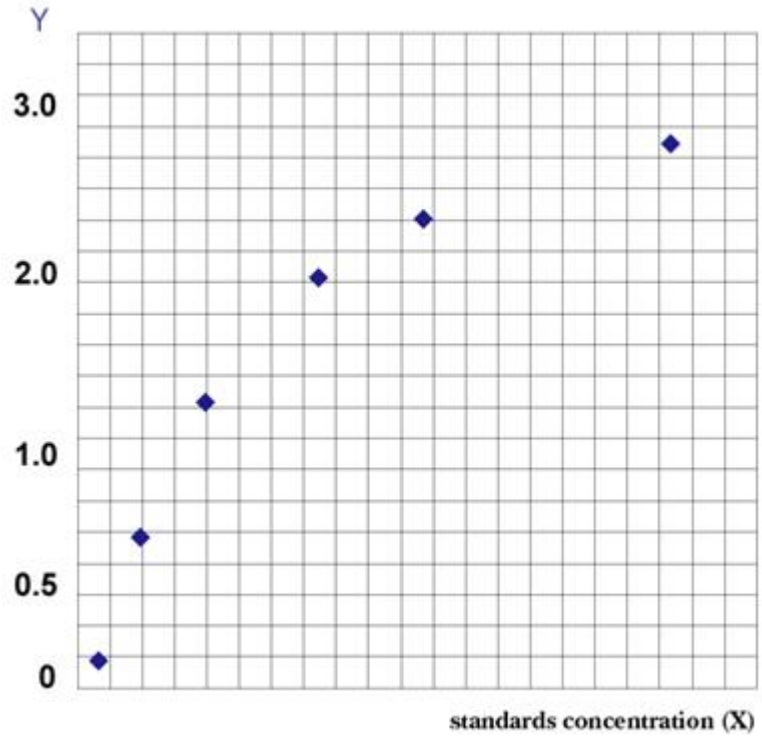
## Assay Procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.
2. Add standard: Set Standard wells, testing sample wells. Add standard 50µl to standard well.
3. Add Sample: Add testing sample 10µl then add Sample Diluent 40µl to testing sample well; Blank well doesn't add anyting.
4. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400µl) 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 Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.
7. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

## Calculation

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of interseation, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

## Typical Standard Curve




## Sensitivity

The sensitivity by this assay is 0.1 ng/ml.

## 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.