Thryoxine (T4) ELISA (Mouse/Rat)

For Research Use Only. Not for use in Diagnostic Procedures.

INTENDED USE

The NeoBiotech Anthrax protective antigen (PA) IgG ELISA Kit is intended for use in the evaluation of sample's immune status or exposure to Anthrax. For research use only.

PRINCIPLES OF THE TEST

Diluted sample serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibodyantigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with PA recombinant antigen	12x8x1
2. Sample Diluent: 1 bottle	22 ml
3. Calibrator	1.5 ml
4. Positive Control	1.5 ml
5. Negative Control	1.5 ml
6. Enzyme Conjugate	12 ml
7. TMB Substrate: 1 bottle	12 ml
8. Stop Solution	12 ml
9. Wash Concentrate 20X	25 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water

2. precision pipettes

3. Disposable pipette tips

4. Micortiter well reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8°C.

2. Keep microwells sealed in a dry bag with desiccants.

3. The reagents are stable until expiration of the kit.

4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. **Potential biohazardous materials:**
   The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.

2. This kit is designed for research use only.

3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum.

2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of samples. months. Avoid repetitive freezing and thawing of samples.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

ASSAY PROCEDURE.

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.

2. Negative control, positive control, and calibrator are ready to use.

3. Prepare 1:41 dilution of test samples, by adding 5 µl of the sample to 200 µl of sample diluent. Mix well.

4. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.

5. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel.

6. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.

7. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel.

8. Dispense 100 µl of TMB substrate solution and incubate for 10 minutes at room temperature.

9. Add 100 µl of Stop Solution to stop reaction.

10. Read O.D. within 15 min at 450 nm using microwell reader.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.

2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).

3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:

Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2.

INTERPRETATION

The following is intended as a guide to interpretation of PA IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.
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Antibody Index Interpretation

<0.9   No detectable antibody to PA IgG by ELISA.
0.9-1.1   Borderline positive. Follow-up testing is recommend if indicated.
>1.1   Indicative of vaccination, current or previous Anthrax infection

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to detection and should be interpreted in relation to the sample history, physical findings and other procedures.

2. Lipemic or hemolyzed samples may cause erroneous results.

PRERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity
52 samples were tested and the results are summarized below:

		—	±	—	Total
Predicate	—	25	10	1	36
	±	0	0	0	0
	—	1	0	69	70

Relative Specificity = 69/70 x 100 = 98%, Relative Sensitivity = 35/36 x 100 = 97%

2. Precision

Intra Assay:

Serum	No. of replicates	Mean	Std Dev	CV%
1	20	1.821	0.094	5.18
2	20	1.095	0.039	3.58
3	20	2.041	0.017	3.69
4	20	1.458	0.045	3.09
5	20	2.309	0.077	3.34

Inter Assay:

Serum	No. of replicates	Mean	Std Dev	CV%
1	16	1.974	0.162	8.22
2	16	1.472	0.113	7.68
3	16	2.293	0.197	8.61
4	16	1.138	0.097	8.54
5	16	1.891	0.133	7.06

REFERENCES

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3. Pittman PR, Hack D, Mangiafico J, Gibbs P, McKee KT Jr, Friedlander AM, Sjogren MH. Antibody response to a delayed booster dose of anthrax vaccine and botulinum toxoid. Vaccine 2002 May 15;20(16):2107-15.

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