**TECHNICAL BULLETIN**

**Product Information**

**Anthrax Protective Antigen IgG ELISA**

Catalog Number SE120147

Storage Temperature 2–8 °C

**Product Description**

*Bacillus anthracis*, the etiologic agent of anthrax, is a large, Gram-positive, nonmotile, spore-forming bacterial rod. The three virulence factors of *B. anthracis* are edema toxin, lethal toxin, and a capsular antigen. Human anthrax has three major clinical forms: cutaneous, inhalation, and gastrointestinal. Cutaneous anthrax is a result of introduction of the spore through the skin; inhalation anthrax, through the respiratory tract; and gastrointestinal anthrax, by ingestion. In the United States, incidence of naturally acquired anthrax is extremely low. Gastrointestinal anthrax is rare but may occur as explosive outbreaks associated with ingestion of infected animals. Worldwide, the incidence is unknown, though *B. anthracis* is present in most of the world. If untreated, anthrax in all forms can lead to septicemia and death. Early treatment of cutaneous anthrax is usually curative and early treatment of all forms is important for recovery. Patients with gastrointestinal anthrax have reported case-fatality rates ranging from 25–75%. Case-fatality rates for inhalational anthrax are thought to approach 90–100%. Because *B. anthracis* has a high probability for use as an agent in biologic terrorism, many centers are involved in studying the epidemiological and laboratory diagnostic of this bacterium. An ELISA test for the detection of IgG antibody to Anthrax Protective Antigen (PA) can be used to study the efficacy of experimental anthrax vaccine and the exposure to this antigen.

The Anthrax Protective Antigen (PA) IgG ELISA Kit is intended for use in the evaluation of patient’s immune status or exposure to anthrax. Diluted 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 oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

**Components**

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with PA recombinant antigen</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle</td>
<td>22 mL</td>
</tr>
<tr>
<td>Calibrator</td>
<td>1 mL</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
</tr>
<tr>
<td>Wash Concentrate 20x</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

**Reagents and Equipment Required but Not Provided.**

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. Multiwell plate reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

**Sample Preparation**

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.

**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).
Storage/Stability
Store the kit at 2–8 °C.

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

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.

Lipemic or hemolyzed samples may cause erroneous results.

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab Index for Positive control should be >1.2.

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 41-fold 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 100 µL of 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 300 µL of 1x wash buffer. Blot on absorbent 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 300 µL of 1x wash buffer. Blot on absorbent 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.
Results
Calculations
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
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

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

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

References

CH,MAM,RGC 10/14-1

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