# DATA SHEET

**Mouse Anti-Anthrax Protective Antigen 83 [PA83]**

**ELISA Kit Cat. No. 900-105-83G**

**For Quantitative Determination of**

**Anti-PA83 IgG (IgG-specific) in Mouse Serum**

INTENDED USE

The Mouse Anti-Anthrax Protective Antigen (PA) ELISA Kit is an in vitro immunoassay for research use for quantification of anti-anthrax PA IgG in mouse serum.

RESEARCH USE OF THE TEST

Anthrax is a zoonotic disease caused by the spore-forming bacterium Baci us anthracis. The disease most commonly occurs in wild and domestic mammals (e.g., cattle, sheep, goats, camels, antelope, and other herbivores). Anthrax occurs in humans when they are exposed to infected animals or tissue from infected animals or when they are directly exposed to B. anthracis or the spores.

Depending on the route of infection, anthrax disease can occur in three forms: cutaneous, gastrointestinal, and inhalation. B. anthracis spores can remain viable and infective in the soil for many years.

B. anthracis has also been manufactured as a biological warfare agent because of the ability of its spores to be transmitted by the respiratory route, the high mortality of inhalation anthrax, and the greater stability of B. anthracis spores compared with other potential biological warfare agents. B. anthracis evades the immune system by producing an anti-phagocytic capsule. In addition, B. anthracis produces three proteins - protective antigen (PA), lethal factor (LF), and edema factor (EF) - that act in binary combinations to form two exotoxins known as lethal toxin and edema toxin. PA and LF form lethal toxin; PA and EF form edema toxin. LF is a protease that inhibits mitogen-activated protein kinasekinase. PA is required for binding and translocating LF and EF into host cells. PA is an 83 kD protein that binds to receptors on mammalian cells and is critical to the ability of B. anthracis to cause disease. After binding to the cell membrane, PA is cleaved to a 63 kD fragment that subsequently binds with LF or EF. LF or EF bound to the 63 kD fragment undergoes receptor-mediated internalization, translocation into the cytosol.

An improved vaccine for livestock, based on a live unencapsulated avirulent variant of B. anthracis, has served as the principal veterinary vaccine. AVA, the only licensed human anthrax vaccine in the United States, is produced by BioPort and is prepared from a cell-free

filtrate of B. anthracis culture that contains no dead or live bacteria. The strain used to prepare the vaccine is a toxigenic, non-encapsulated strain known as V770-NP1-R. The filtrate contains a mix of cellular products including PA83 and is adsorbed to aluminum hydroxide as adjuvant. The amount of PA and other proteins per 0.5mL dose is unknown, and all three toxin components (LF, EF, and PA) are present in the product. The efficacy of AVA is based on several studies in animals, one controlled vaccine trial in humans, and immunogenicity data for both humans and lower mammalian species. Approximately 95% of vaccinees seroconvert with a fourfold rise in antiPA IgG titers after three doses. However, the precise correlation between antibody titer (or concentration) and protection against infection is not defined.

While having some efficacy in protecting against anthrax, the dosage, safety and efficacy of this licensed vaccine is being debated by the Scientists as well as politicians. More advanced vaccines are based upon recombinant purified PA83 proteins (Vaxgen).

CALCULATION OF RESULTS (continued)

II. Antibody Activity Units (Titer)

When the dilution curves of samples are not parallel to the Standard curve, antibody potency can be expressed in semi-quantitative activity units, using one of the Standards as the Index:

1. Calculate the mean net ODs for replicate samples and the selected Standard.

2. Divide each sample OD value by the Standard OD value, and multiply by the sample dilution and the Standard (U/ml) value = Total Activity Units Typical Results: see Data Table in Section I.

0.92 [Sample, net OD] ÷ 1.22 [250 U/ml Std, net OD]

x 1000 dilution x 250 U/ml = 189k Activity Units in serum.

ASSAY CHARACTERISTICS

Specificity

The coated antigen used in this kit is purified recombinant Bacillus anthracis protective antigen 83; thus the assay is specific for antibodies directed to PA83.

Antibodies generated to PA63 and PA20 may bind to the recombinant full length PA83 as well. The anti-Mouse IgG HRP conjugate reacts with mouse IgG (gamma chain specific) antibodies that bind to PA83 on the plate. IgE, IgA or IgM antibody would not be measured above background signals.

Standard Values

The standards are composed of mouse-anti-PA83 IgG (ADI reference). Values are assigned as arbitrary anti-PA83 activity units (see Limits of the Assay). 1000 U/ml is equal to 200 ng/ml of the mouse IgG.

Precision

Samples containing low, medium and high concentrations of anti-PA83 were assayed as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

Anti-PA83 IgG concentrations were measured with very good between-assay (5.8 to 7.5 %CV) reproducibility.

| Sample | Anti-PA83 U/ml | Inter-assay %CV |
|---|---|---|
| Low PA83 | 258 | **7.2** |
| Medium PA83 | 620 | **5.8** |
| High PA83 | 1030 | **7.5** |

CALCULATION OF RESULTS

I. Use of a Standard Curve

When the dilution curves of samples are parallel to the Standard curve (see Limits of the Assay), the anti-PA83 activity units may be determined by interpolation from the Standard curve, as follows:

1. The results may be calculated using any immunoassay software package. The fourparameter curve-fit is recommended. If software is not available, anti-PA83 activity concentrations may be determined as follows:

2. Calculate the mean OD of duplicate samples.

3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (U/ml) of anti-PA83 (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.

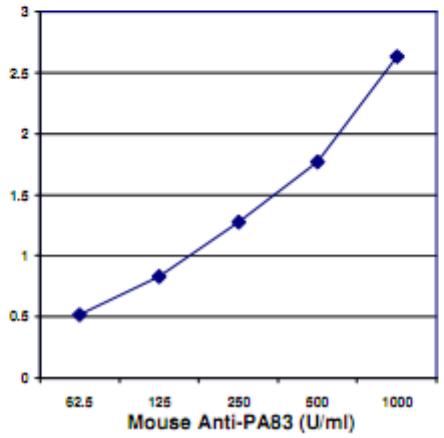
4. The anti-PA83 activity concentrations in unknown samples and controls can be determined by interpolation from the standard curve.

5. Multiply the values obtained for the samples by the dilution factor of each sample.

6. Samples producing signals higher than the 1000 U/ml standard should be further diluted and re-assayed.

**Typical Results:**

| Wells | Standards & Samples | A450 nm | x-PA U/ml |
|---|---|---|---|
| A1, A2 | **Negative Diluent Control** | 0.03 | 0 |
| B1, B2 | 62.5 U/ml **Standard** | 0.52 | 62.5 |
| C1, C2 | 125 U/ml **Standard** | 0.83 | 125 |
| D1, D2 | 250 U/ml **Standard** | 1.28 | 250 |
| E1, E2 | 500 U/ml **Standard** | 1.77 | 500 |
| F1, F2 | 1000 U/ml **Standard** | 2.63 | 1000 |
| G1, G2 | **Positive Control** [Value: 245 – 455 ng/ml] | 1.48 | 330 |
| H1, H2 | **Sample** [Diluted 1:1000] | 0.98 | 174 |
| | Calculated: 1000-fold dilution x 174 U/ml = **174 kU**/ml in serum | | |




PRINCIPLE OF THE TEST

The Mouse Anti-Anthrax PA83 ELISA kit is based on the binding of Mouse anti-Anthrax PA83 in samples to recombinant anthrax PA83 antigen immobilized on the microtiter wells.

After a washing step, anti-Mouse IgG-HRP conjugate is added, which binds to anti-Anthrax PA from the sample bound to the coated anthrax PA. After another washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of anti-Anthrax PA83 present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of anti-Anthrax PA83 in samples and control is calculated from a curve of standards containing assigned activity units of mouse anti-Anthrax PA antibody.

KIT CONTENTS

To Be Reconstituted: Store as indicated.

| Component | Instructions for Use |
|---|---|
| **Sample Diluent Concentrate (20x)** Cat. No. SD-20T, 10ml | Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up. |
| **Wash Solution Concentrate (100x)** Cat. No. WB-100, 10ml | Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely. |
| **Anti-Mouse IgG HRP Conjugate Concentrate (100x)** Part No. MsH-G, 0.25ml | Peroxidase conjugated anti-Mouse IgG in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage. |

**Ready For Use:** Store as indicated on labels.

| Component | Part No. | Amt | Contents |
|---|---|---|---|
| **PA83 Microwell Strip Plate** | 900-101 | 8-well strips (12) | Coated with recombinant PA83, and post-coated with stabilizers. |
| **Mouse Anti-Anthrax PA83 Standards** | | | |
| 62.5 U/ml<br>125 U/ml<br>250 U/ml<br>500 U/ml<br>1000 U/ml | 900-103B<br>900-103C<br>900-103D<br>900-103E<br>900-103F | 1.0 ml<br>1.0 ml<br>1.0 ml<br>1.0 ml<br>1.0 ml | Five (5) vials, each containing calibrated Mouse anti-PA83 IgG; diluted in buffer with protein, detergents and ProClin 300 as stabilizers. |
| **Positive Control** [U/ml] range on label | 900-102 | 1.0 ml | Mouse anti-PA83 IgG with stated concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers. |
| **TMB Substrate** | 80091 | 12 ml | Chromogenic substrate for HRP containing TMB and peroxide. |
| **Stop Solution** | 80101 | 12 ml | 1% sulfuric acid. |

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.

Disposable glass or plastic 5-15ml tubes for diluting samples and antiMouse IgG HRP Concentrate.

- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.

- Stock bottle to store diluted Wash Solution; 200ml to 1L.

- Distilled or deionized water to dilute reagent concentrates.

- Microwell plate reader at 450 nm wavelength.

## PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and anti-Mouse IgG-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

## SPECIMEN COLLECTION AND HANDLING

Serum and other biological fluids may be used as samples with proper dilution to

avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8 oC until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

## ASSAY PROCEDURE

Bring all reagents to room temperature (25-30 oC) equilibration (at least 30 minutes).

Dilute samples in Working Sample Diluent according to expected anti-PA83 levels; dilute at least 200-fold (e.g., 5ul sample + 995 ul Diluent) to reduce nonspecific signals.

DO NOT dilute the Standards or Control.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

**1. Set-up**

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

**2. 1st Incubation [100 ul -60min; 4 washes]**

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

**3. 2nd Incubation [100 ul - 30min; 5 washes]**

- Add 100ul of Working Anti-Mouse IgG HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

**4. Substrate Incubation [100 ul - 15min]**

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read the final OD at 405-410 nm (signals will be lower, but data is accurate).

**5. Stop Step [Stop: 100ul]**

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

**6. Absorbance Reading**

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

LIMITS OF THE ASSAY

Quantitation of Antibody in a Sample The ELISA measures anti-PA83 activity, a combination of antibody concentration and avidity for the PA83 antigen. Antibodies with substantially different specific IgG concentrations may display similar anti- PA83 activities, due to differences in avidity. The quantitation or potency of the samples is, therefore, appropriately expressed in activity Units (titer), rather than mass units of IgG (e.g., ug/ml).

Standard Curve Quantitation

To quantitate antibody activity from a standard curve (such as provided with the kit), the dilution curve of the samples must be parallel to the standard curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in antigen avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as a titer relative to a reference positive such as the 250 U/ml Standard, or another Standard in the kit (see Calculation of Results).

| ELISA Kit Components | Amount | Part No. |
|---|---|---|
| Anthrax PA83 Microwell Strip Plate, 8-well strips (12) | 96 wells | 900-101 |
| Ms anti-PA83 Positive Control [value range on the vial] | 1.0 ml | 900-102 |
| Ms anti-PA83 IgG Standard 62.5 U/ml | 1.0 ml | 900-103B |
| Ms anti-PA83 IgG Standard 125 U/ml | 1.0 ml | 900-103C |
| Ms anti-PA83 IgG Standard 250 U/ml | 1.0 ml | 900-103D |
| Ms anti-PA83 IgG Standard 500 U/ml | 1.0 ml | 900-103E |
| Ms anti-PA83 IgG Standard 1000 U/ml | 1.0 ml | 900-103F |
| Anti-Mouse IgG HRP Conjugate (100X) | 0.25 ml | MsH-G |
| Sample Diluent Concentrate (20X) | 10 ml | SD-20T |
| Wash Solution Concentrate (100X) | 10 ml | WB-100 |
| TMB Substrate | 12 ml | 80091 |
| Stop Solution | 12 ml | 80101 |
| Product Manual | 1 ea | M-900-105-83G |

Standard in the kit (see Calculation of Results).

| Catalog# | ProdDescription |
|---|---|
| 900-100-83T | Mouse Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 900-105-83G | Mouse Anti- PA83 IgG-specific) ELISA kit |
| 900-120-83T | Rabbit Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 900-130-83T | Goat Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 900-140-83T | G. pig Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 900-150-83T | Monkey Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 900-160-83T | Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 900-200-LFM | Mouse Anti-lethal Factor (LF) Ig's ELISA kit |
| 900-220-LFR | Rabbit Anti-Edema Factor (EF) Ig's ELISA kit |
| 900-300-EFR | Mouse Anti-Edema Factor (EF) Ig's ELISA kit |
| 900-320-EFR | Rabbit Anti-Edema Factor (EF) Ig's ELISA kit |
| 800-100-P83 | Anthrax Protective Antigen 83 (PA83) Protein ELISA Kit |

Recombinant Proteins (PA83, PA63, PA20, EF, LF, EF) and antibodies are also available.



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