QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls Each lab should assay internal positive control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard.

ELISA Kit Components	Amount	Part No.
Anti-Anthrax PA83 Microwell Strip Plate	8-well strips (12)	800-101
Anthrax PA83 Standard 20 ng/ml	1.0 ml	800-103A
Anthrax PA83 Standard 50 ng/ml	1.0 ml	800-103B
Anthrax PA83 Standard 100 ng/ml	1.0 ml	800-103C
Anthrax PA83 Standard 200 ng/ml	1.0 ml	800-103D
Anthrax PA83 Standard 400 ng/ml	1.0 ml	800-103E
Anthrax PA83 Standard 1000 ng/ml	1.0 ml	800-103F
Anti-Anthrax PA83 HRP Conj. (100X)	0.25 ml	800-104
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-800-100-P83

Instruction Manual No. M-800-100-P83

Anthrax Protective Antigen 83 ELISA Kit

Cat. No. 800-100-P83, 96 tests

For Quantitative Determination of Anthrax Protective Antigen (PA83) in vaccines or biological buffers

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
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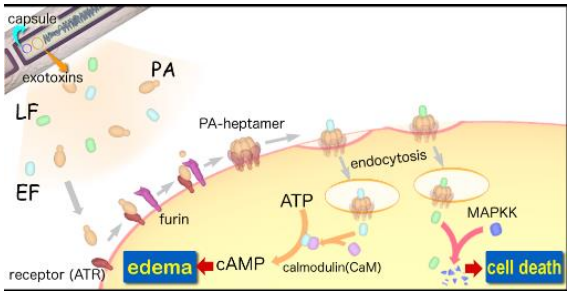
INTENDED USE

The Anthrax Protective Antigen (PA83) ELISA Kit is an in vitro immunoassay for research use for quantification of PA83 in cell culture media, recombinant preparations or vaccines or other fluids containing PA83. The kit contains no active (live or killed) or bacterial proteins to avoid infection. This kit is for research use only (RUO) and not for diagnosis cure or prevention of the disease.

RESEARCH USE OF THE TEST

Anthrax is a zoonotic disease caused by the spore-forming bacterium *Bacillus 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 kinase-kinase. PA is required for binding and translocating LF and EF into host cells. PA is an 83 kD (**PA83**) 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 (**PA63**) 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. However, the use of livestock vaccines was associated with occasional animal casualties, and live vaccines were considered unsuitable for humans. 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 anti-PA IgG titers after three doses. However, the precise correlation between antibody titer (or concentration) and protection against infection is not defined. More advanced vaccines are based upon recombinant purified PA83 proteins (Vaxgen).

ASSAY CHARACTERISTICS

Specificity
The antibodies used in this kit have been affinity purified using a purified recombinant protective antigen (PA83) immunosorbent and have been shown by ELISA to react specifically with PA, and to have no reactivity with recombinant lethal factor (LF) or edema factor (EF).

Value-assignment of Standards
The purified recombinant protective antigen used for standards represented 100% of the protein on an SDS-PAGE gel, with an apparent molecular weight of 83,000 daltons. Protein concentration was assigned using a Bradford modified assay with BSA as standard.

Precision
Samples containing low, medium and high concentrations of 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.

PA83 concentrations were measured with good between-assay (7.8 to 9.7 %CV) reproducibility.

Sample	PA83 ng/ml	Inter-assay %CV
Low PA83	24.7	**9.7**
Medium PA83	60.6	**8.4**
High PA83	71.8	**7.8**

Related Items

Catalog#	Product Description
800-100-RT-25	Anthrax Protective Antigen 83 (PA83) Protein Rapid test cards (results in 2-10 mins),
800-120-LF	Anthrax Lethal Factor (LF) Protein ELISA Kit, 96 tests
800-130-EF	Anthrax Edema Factor (EF) Protein ELISA Kit, 96 tests
900-100-83T	Mouse Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit, 96 tests, Quantitative
900-105-83G	Mouse Anti-Anthrax Protective Antigen 83 (PA83) IgG-specific) ELISA kit, 96 tests
900-110-83G	Bovine/Cow Anti-Anthrax Protective Antigen 83 (PA83) IgG-specific) ELISA kit, 96 tests
900-115-83G	Swine/Pig/Porcine Anti-Anthrax Protective Antigen 83 (PA83) IgG-specific) ELISA kit, 96 tests
900-120-83T	Rabbit Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit,96 tests
900-130-83T	Goat Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit, 96 tests
900-135-83G	Deer/Elk Anti-Anthrax Protective Antigen 83 (PA83) IgG ELISA kit, 96 tests
900-140-83T	G. pig Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit, 96 tests
900-150-83T	Monkey Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit, 96 tests
900-165-83G	Human Anti-Anthrax Protective Antigen 83 (PA83) IgG ELISA kit, 96 tests
900-170-83G	Sheep Anti-Anthrax Protective Antigen 83 (PA83) IgG ELISA kit, 96 tests
900-200-LFM	Mouse Anti-Anthrax Lethal Factor (LF) Ig's ELISA kit, 96 tests
900-210-LFH	Human Anti-Anthrax Lethal Factor (LF) IgG ELISA kit, 96 tests
900-220-LFR	Rabbit Anti-Anthrax Lethal Factor (LF) Ig's ELISA kit, 96 tests
900-230-LFG	Swine/Feral Anti-Anthrax Lethal Factor (LF) IgG ELISA kit, 96 tests
900-235-LFM	Swine/Feral Anti-Anthrax lethal Factor (LF) IgM ELISA kit, 96 tests
900-240-LFB	Bovine Anti-Anthrax lethal Factor (LF) IgG ELISA kit, 96 tests
900-270-LFD	Deer/Elk Anti-Anthrax Lethal Factor (LF) IgG ELISA kit, 96 tests
900-280-LFM	Monkey Anti-Anthrax Lethal Factor (LF) IgG ELISA kit, 96 tests
900-300-EFM	Mouse Anti-Anthrax Edema Factor (EF) Ig's ELISA kit, 96 tests
900-310-EFH	Human Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests
900-320-EFR	Rabbit Anti-Anthrax Edema Factor (EF) Ig's ELISA kit, 96 tests
900-330-EFG	Swine/Feral Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests
900-335-EFM	Swine/Feral Anti-Anthrax Edema Factor (EF) IgM ELISA kit, 96 tests
900-340-EFB	Bovine Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests
900-350-EFG	Goat Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests
900-360-EFS	Sheep Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests
900-370-EFD	Deer/Elk Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests
900-380-EFM	Monkey Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests

CALCULATION OF RESULTS

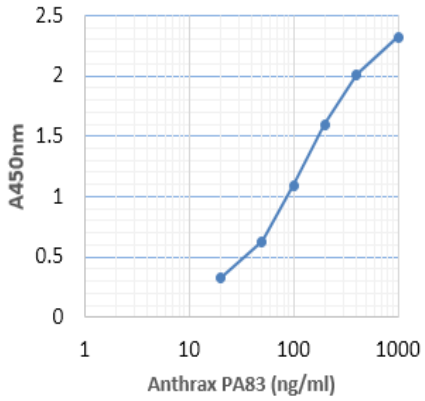
1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, PA83 concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of 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 PA83 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 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	PA83 ng/ml
A1, A2	**Negative Diluent Control**	0.05	
B1, B2	20 ng/ml **Standard**	0.32	
C1, C2	50 ng/ml **Standard**	0.63	
D1, D2	100 ng/ml **Standard**	1.09	
E1, E2	200 ng/ml **Standard**	1.60	
F1, F2	400 ng/ml **Standard**	2.01	
G1, G2	1000 ng/ml **Standard**	2.32	
H1, H2	**Sample** [Diluted 1:20]	1.85	305
	Calculated: 20-fold dilution x 305 ng/ml = **6.10** ug/ml in sample		

A typical assay Standard Curve (do not use for calculating sample values)



/BG-Graph-1

PRINCIPLE OF THE TEST

The Anthrax PA83 ELISA kit is based on the binding of PA83 in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a 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 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 PA83 in samples and control is calculated from a curve of standards containing known concentrations of recombinant PA83.

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 2-8ºC 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-PA83 HRP Conjugate Concentrate (100x) Part No. 800-104, 0.25ml	Peroxidase conjugated anti-PA83 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 2-8ºC storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-PA83 coated Strip Plate	640-211	8-well strips (12)	Coated with anti-PA83, and post-coated with stabilizers.
Anthrax PA83 Standards			
20 ng/ml	800-103A	1.0 ml	Six (6) vials, each containing calibrated recombinant PA83; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
50 ng/ml	800-103B	1.0 ml	
100 ng/ml	800-103C	1.0 ml	
200 ng/ml	800-103D	1.0 ml	
400 ng/ml	800-103E	1.0 ml	
1000 ng/ml	800-103F	1.0 ml	
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 pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and anti-PA83 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, Sample Diluent, and anti-PA83-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

Culture medium, buffers and other fluids may be used as samples with proper dilution to avoid solution matrix interference and adjustment of pH to 6-8. In all cases, 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^{O}C 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 (18-30^{o}C) equilibration (at least 30 minutes).

Dilute samples in Working Sample Diluent according to expected PA83 concentrations; dilute at least 5-fold (e.g., 100ul sample + 400 ul Diluent) to reduce nonspecific signals.

DO NOT dilute the Standards.

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 [100ul -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 is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

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

- Add 100ul of Working Anti-PA83 HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul - 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, assuring the top standard does not surpass 2 OD.

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 using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.