

**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. Do not rely on results generated from an assay with these issues.

*Instruction Manual No. M-900-120-83T*

ELISA Kit Components	Amount	Part No.
Anthrax PA83 Microwell Strip Plate, 8-well strips (12)	96 wells	900-101
Anti-Anthrax PA83 Positive Control [lot specific concentration on the vial]	1.0 ml	900-122
Rabbit Anti- PA83 Standard 10 U/ml	1.0 ml	900-123B
Rabbit Anti- PA83 Standard 20 U/ml	1.0 ml	900-123C
Rabbit Anti- PA83 Standard 40 U/ml	1.0 ml	900-123D
Rabbit Anti- PA83 Standard 80 U/ml	1.0 ml	900-123E
Rabbit Anti- PA83 Standard 160 U/ml	1.0 ml	900-123F
Anti-Rabbit Ig -HRP Conjugate (100X)	0.25 ml	900-124
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
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## Rabbit Anti-Anthrax Protective Antigen 83 (PA83)

ELISA Kit Cat. No. 900-120-83T

**For Quantitative Determination of Total Antibodies to Anthrax PA83 in Rabbit Serum**



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### Related items

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
900-170-83WM	Mouse Anti-Anthrax Protective Antigen 83 (PA83) Ig's WB kit, 12 tests
900-180-83WR	Rabbit Anti-Anthrax Protective Antigen 83 (PA83) Ig's WB kit, 12 tests
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.

For more details please consult our web site ([www.4adi.com](http://www.4adi.com)) or contact us by email ([service@4adi.com](mailto:service@4adi.com))

## INTENDED USE

The Rabbit Anti-Anthrax Protective Antigen (PA) ELISA Kit is an in vitro immunoassay for research use for quantification of total anti-anthrax PA Ig's in rabbit serum.

## 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. 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 anti-PA 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).

## ASSAY CHARACTERISTICS

### Specificity

The coated antigen used in this kit is purified recombinant *Bacillus anthracis* protective antigen 83. The antibody conjugate used in the kit is directed against the rabbit IgG (H+L) chains and, therefore, anthrax PA83-specific antibodies of primarily IgG are measured; IgM, and IgA subtypes may be detected and contribute to total antibody activity. Antibodies generated to PA63 and PA20 may bind to the recombinant full length PA83 as well. It is possible to change the conjugate to anti-rabbit IgG (Fc or heavy chain specific) or anti-IgM to detect only the IgG or IGM antibodies respectively. ADI supplies these conjugates separately.

### Value-assignment of Standards

The anti-PA used for standards represents rabbit anti-PA83 Ig's, and assigned activity concentration in arbitrary units, U/ml (ADI reference serum).

### 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 Ig 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	20.6	7.2
Medium PA83	49.6	5.8
High PA83	98.4	7.5

## 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.

## CALCULATION OF RESULTS

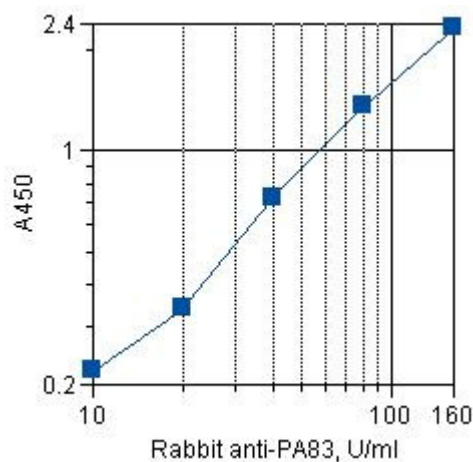
- The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, PA83 concentrations may be determined as follows:
- Calculate the mean OD of duplicate samples.
- On graph paper plot the mean OD of the standards (y-axis) against the concentration (U/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.
- The PA83 concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
- Multiply the values obtained for the samples by the dilution factor of each sample.
- Samples producing signals higher than the 160 U/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	PA-Ig U/ml
A1, A2	<b>Negative Diluent Control</b>	0.05	0
B1, B2	10 U/ml <b>Standard</b>	0.22	10
C1, C2	20 U/ml <b>Standard</b>	0.34	20
D1, D2	40 U/ml <b>Standard</b>	0.72	40
E1, E2	80 U/ml <b>Standard</b>	1.37	80
F1, F2	160 U/ml <b>Standard</b>	2.35	160
G1, G2	<b>Positive Control</b> [Value: 39 – 73 U/ml]	0.95	51
H1, H2	<b>Sample</b> [Diluted 1:1000] Calculated: 1000-fold dilution x 52 U/ml = 52 kU/ml in serum	0.96	52

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



## PRINCIPLE OF THE TEST

The Rabbit Anti-Anthrax PA83 ELISA kit is based on the binding of rabbit anti-Anthrax PA83 Ig's in samples to recombinant anthrax PA83 antigen immobilized on the microtiter wells. After a washing step, anti-rabbit Ig-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 concentrations of rabbit anti-Anthrax PA calibrated in activity units.

## KIT CONTENTS

**To Be Reconstituted:** Store as indicated.

Component	Instructions for Use
<b>Sample Diluent Concentrate (20x)</b> Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as <b>Working Sample Diluent</b> and store at 2-8°C until the kit lot expires or is used up.
<b>Wash Solution Concentrate (100x)</b> Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as <b>Working Wash Solution</b> and store at ambient temperature until kit is used entirely.
<b>Anti-Rabbit Ig - HRP Conjugate Concentrate (100x)</b> Part No. 900-124, 0.25ml	Peroxidase conjugated anti-rabbit IgG (h&l) in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of <b>Working Sample Diluent</b> 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
<b>PA83 Microwell Strip Plate</b>	900-101	8-well strips (12)	Coated with recombinant PA83, and post-coated with stabilizers.
<b>Rabbit Anti-Anthrax PA83 Standards</b>			
10 U/ml	900-123B	1.0 ml	Five (5) vials, each containing rabbit anti-PA83 antibodies, calibrated in anti-PA83 activity units, U/ml; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
20 U/ml	900-123C	1.0 ml	
40 U/ml	900-123D	1.0 ml	
80 U/ml	900-123E	1.0 ml	
160 U/ml	900-123F	1.0 ml	
<b>Positive Control</b> [Anti-PA83] range on label	900-122	1.0 ml	Rabbit anti-PA83 with stated activity range, U/ml; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
<b>TMB Substrate</b>	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
<b>Stop Solution</b>	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 anti-rabbit Ig 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-rabbit Ig-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°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 (25-30° C) 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. 1<sup>st</sup> Incubation [100ul -60 min; 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. 2<sup>nd</sup> Incubation [100ul – 30 min; 5 washes]

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

#### 4. Substrate Incubation [100ul – 15 min]

- 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.