ELISA Kit Components	Amount	Cat/Part No.
Anti-Protein A Microwell Strip Plate	8-well strips (12)	800-111
Protein A Stock, lyophilized	2 vials	800-112
Anti-Protein A HRP Conjugate	0.15 ml	800-113
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-800-110

RELATED MATERIAL available from ADI

Catalog #	Description
800-120-PRG	Protein G ELISA Kit
PRTA55	Recombinant Protein A Coated Microplates
PRTG55	Recombinant Protein G Coated Microplates
PRTA11-R	Recombinant purified Protein A
PRTA12-A	Anti-Protein A IgG
PRTA13-A	Anti-Protein A IgG, aff pure
PRTA13-A	Anti-Protein A IgG-biotinylated
PRTA13-A	Anti-Protein A IgG-HRP Conjugate
PRTG15-R	Recombinant purified Protein G
PRTA15-AS	Recombinant Protein A - Agarose

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-800-110

Protein A

ELISA Kit Cat. No. 800-110-PRA

For Quantitative Determination of Protein A in Solutions

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

INTENDED USE

The Protein A ELISA Kit is an in vitro immunoassay for the highly sensitive detection and/or quantification of Protein A in fluids. The kit is designed to measure trace contamination of Protein A in antibody solutions.

DISCUSSION

Staphlococcus aureus Protein A (Pro A) is a cell wall immunoglobulin (IgG)-binding protein that is secreted by essentially all clinical isolates of *S. aureus*. The molecule binds to the Fc region of most mammalian IgGs; because of this property, Pro A's isolated native form or recombinant truncated form has been used in many laboratory and commercial applications for the detection and purification of antibodies.

Pro A immunosorbents are widely used for purification of monoclonal and polyclonal antibodies. Possible leakage of Pro A during processing may contaminate the purified antibody preparations. The detection/measurement of contaminating Pro A is complicated by Pro A-IgG binding in the sample, which inhibits the subsequent binding of the Pro A to the ELISA antibodies required for accurate quantification. A sample pretreatment boiling step for Pro A samples containing mouse IgG heat denatures the IgG. This uncouples the Pro A-IgG binding and, thus, allows for accurate Pro A measurement (see the Sample Recovery section, page 7). Appropriate recovery of Pro A in solutions of IgG from other species has not been elucidated with this ELISA. In these cases, the investigator should demonstrate requirements for appropriate Pro A recovery.

PRINCIPLE OF THE TEST

The Protein A ELISA kit is based on the binding of Protein A 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 Protein A 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 Protein A in samples is calculated from a curve of standards containing known concentrations of Protein A.

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.

PERFORMANCE CHARACTERISTICS

Specificity
The antibodies used in this kit were produced by immunization with recombinant Protein A. Native and recombinant Protein A are quantified in this assay with similar potency.

Sample Recovery
High and low concentrations of Protein A were mixed into buffer containing mouse IgG at 1mg/ml. Samples were assayed before and after a boiling pretreatment step. Observed assay values in samples containing IgG compared to non-IgG sample values ranged from 41 to 62%. With a boiling pretreatment step, observed IgG sample assay values were 75 to 106% of non-IgG values, indicating accurate quantification of Protein A in the presence of IgG using the pretreatment step.

Run	Sample Pretreatment	Pro A + Buffer	Pro A + Mouse IgG	
		Pro A pg/ml	Pro A pg/ml	% *
1	None	2000	1240	62
	Boil	2000	1600	**80**
	None	500	250	50
	Boil	500	490	**98**
2	None	2000	811	41
	Boil	2000	1500	**75**
3	Boil	1100	950	**86**
	Boil	690	730	**106**

* % Recovery: [Pro A + IgG value/ Pro A + Buffer (non-IgG) value] x 100

PRECAUTIONS AND SAFETY INSTRUCTIONS

Sample Diluent and anti-Protein A-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.

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, Protein A concentrations may be determined as follows:

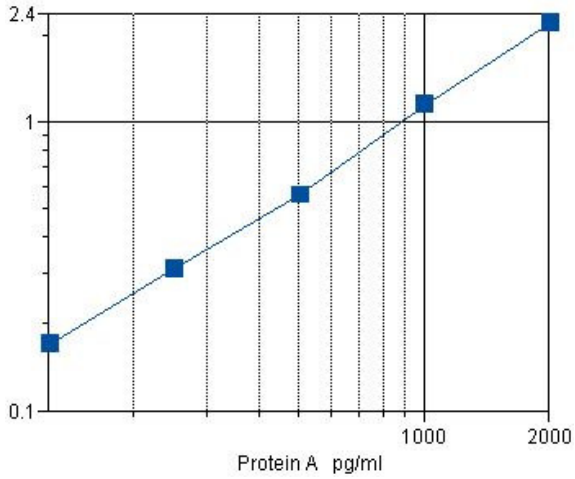
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (pg/ml) of Protein A (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Protein A concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 2000 pg/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	Protein A pg/ml
A1, A2	**Negative Diluent Control**	0.07	0
B1, B2	125 pg/ml **Standard**	0.17	125
C1, C2	250 pg/ml **Standard**	0.31	250
D1, D2	500 pg/ml **Standard**	0.56	500
E1, E2	1000 pg/ml **Standard**	1.15	1000
F1, F2	2000 pg/ml **Standard**	2.21	2000
G1, G2	**Sample** [Diluted 1:4]	0.87	720
	Calculated: 4-fold dilution x 720 pg/ml = **2.88** ng/ml in stock		

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



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** (WS 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 5ml + 495ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely. Add the 100x Concentrate to samples as needed (see **Sample Collection & Handling**, page 3)
Anti-Protein A - HRP Conjugate Concentrate (100x) Part No. 800-113, 0.15ml	Peroxidase conjugated anti-Protein A 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.
Protein A Stock Part No. 800-112	Two (2) vials, each containing Protein A lyophilized in buffer with protein, detergents and ProClin 300 as stabilizers. Keep lyophilized vials refrigerated until used or kit lot expires.

Reconstitute 1 vial with 1.0ml **Working Sample Diluent** to provide a 4000 pg/ml Stock, sufficient for two curves, as diluted below. If samples contain IgG and require a boiling pretreatment, pretreat the Protein A Stock (see Sample Collection & Handling, page 3), then prepare 2-fold dilutions, as follows:

Standard	+	Diluent	=	Final Concn
Reconstituted Stock		None		4000 pg/ml
250 ul of 4000pg/ml		250ul		2000 pg/ml
250 ul of 2000pg/ml		250ul		1000 pg/ml
250 ul of 1000pg/ml		250ul		500 pg/ml
250 ul of 500pg/ml		250ul		250 pg/ml
250 ul of 250pg/ml		250ul		125 pg/ml

Use within two weeks of preparation. Store @ 4C.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Protein A Microwell Strip Plate	800-111	8-well strips (12)	Coated with purified anti-Protein A antibodies.
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 1-15ml tubes for diluting samples, anti-Protein A-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.

For Sample Pretreatment (Samples containing IgG):

- Microfuge or centrifuge
- Conical vials, snap-top, 500ul (or larger)
- Boiling water bath or heat block

SPECIMEN COLLECTION & HANDLING

Sample Set-up

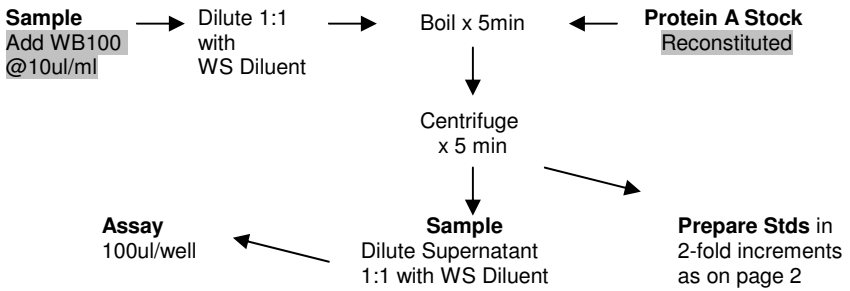
1. Samples must be neutralized to pH 6-8 prior to assay.
2. Protein A readily binds to plastic and glass surfaces in the absence of detergent. Use the Wash Solution Concentrate (100X), WB-100 in the kit, to add 10ul per ml of freshly collected sample and, thus, providing sufficient Tween 20 to stabilize the Protein A.
3. For samples **without** mouse IgG, dilute the sample at least 4-fold in Working Sample Diluent (WS Diluent) and continue directly to the Assay Procedure.

Boiling Pretreatment

For samples **containing** IgG, prepare the sample, along with the reconstituted Protein A Stock, in the following manner:

1. Measure IgG concentration in the sample (e.g., A280 nm: 1.4 OD = 1mg IgG/ml). Dilute the sample with neutral buffer to reduce the IgG concentration below 2 mg/ml. Further dilute the sample 1:1 with Working Sample Diluent.
2. Transfer 300-500ul of each sample and the Protein A Stock (reconstituted) to individual 500ul snap-top microfuge tubes, properly labeled.
3. Place vials in boiling water for 5 minutes, then remove vials and allow cooling.
4. Centrifuge the vials for 5 minutes in a microfuge (high speed), or in a centrifuge at sufficient rpm to precipitate denatured IgG and particulates.
5. Dilute each **Sample** supernatant 1:1 with WS Diluent; dilute the **Protein A Stock** as shown on page 2. Proceed to Assay Procedure.

Boiling Pretreatment



ASSAY PROCEDURE

Bring all reagents to lab temperature (18-30° C) equilibration (at least 30 minutes).

Samples undergoing the boiling pretreatment shown on page 3 have been diluted 4-fold by the end of the pretreatment. Dilute samples that are not pretreated at least 4-fold in Working Sample Diluent prior to the assay; for example, 100ul Sample + 300ul Diluent. Dilute Standards according to Reconstitution instructions on page 2.

PERFORM ALL STEPS AT LAB 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 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 prior to sample addition.
- Aspirate or dump the liquid and pat the strips dry on a paper towel.

2. 1st 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 maybe used. Improper washes may lead to falsely elevated signals and poor reproducibility.

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

- Add 100ul of Working Anti-Protein A-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, or read OD at 405-410 nm (results are valid).

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, use a program to subtract OD at 630nm to normalize well background.