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Cat. No. S-9067
Protein A ELISA kit

SPECIFICATION

- **Assay time:** 160 minutes, total
- **Assay range:** 0 - 2 ng/ml
- **Assay linear range:** 0.05 - 2.0 ng/ml
- **Absorbance value (minimum and maximum limits):**
  a. minimum absorbance: < 0.1 OD at 0 ng/ml protein A
  b. maximum absorbance: 3 OD at 2 ng/ml protein A with no IgG (standard protocol) or 50 ng/ml (shortened protocol)
- **Precision:**
  a. Intra-assay CV < 5%
  b. Inter assay CV < 10%
- **Shelf-life:** 1 year
- **Storage:** 4 °C
- **Kit size:** 96 single determinations including controls

COMPONENTS

1 Microtiter plate (12 x 8 well strips) coated with anti-protein A antibody	
1 Protein A standard	20 ng/ml, 10x, 0.3 ml
1 Human IgG, protein A-free	10 mg/ml, 0.5 ml
1 Biotinylated anti-protein A,	11 ml
1 Strepavidin-HRP	11 ml
1 TMB substrate solution	15 ml
1 stop solution	15 ml, contains 2N sulfuric acid
1 Wash buffer (20x)	60 ml, contains 1% Tween 20

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BACHEM

Protein A ELISA

For the Detection and Quantitation of Staphylococcal Protein A

Sensitivity: 50 - 2,000 pg/mL

For Research Use Only

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BACKGROUND

Protein A is a 42 kD constituent of the bacterial cell wall of *Staphylococcus aureus*[1] and is known for its binding affinity for the Fc portion of most mammalian IgG. Protein A is therefore widely used for both the detection and the purification of such antibodies[2], including IgG for therapeutic use. Contamination of therapeutic IgG preparations with Protein A can cause anaphylactic reactions in sensitized individuals[1], or assay interference in IgG reagents. The Peninsula Laboratories Inc. Protein A ELISA kit provides a sensitive method for measuring Protein A in aqueous solutions. A specific protocol is included for measuring Protein A contamination in IgG preparations, which is essential for confirming their purity.

PROTEIN A RECOVERY IN THE PRESENCE OF IgG

The reactivity of Protein A with the Fc portion of IgG from most mammalian species precludes using mammalian polyclonal antibodies as Protein A assay reagents. However, chicken IgY does not have Fc-reactivity to Protein A, and its use in the Peninsula Laboratories Inc. Protein A ELISA kit overcomes this problem.

Additionally, the epitopes required to detect Protein A by immunoassay are normally blocked when Protein A is bound to IgG. Accurate detection of Protein A in such specimens requires that the standard reference Protein A be prepared in the same concentration of IgG that is present in the samples, and that both the standards and samples be boiled prior to running the assay to denature and precipitate the interfering IgG.

Finally, Tween 20 must be present in the sample to prevent loss of Protein A due to adsorption to glass or plastic surfaces. If neutralization buffer does not contain Tween 20, then 20X Wash Buffer Concentrate can be added to bring the final Tween 20 concentration to 0.05%.

ASSAY DESCRIPTION

The Peninsula Laboratories Inc. Protein A assay has been optimized to overcome IgG interference and to provide accurate measurement of Protein A in samples containing either no IgG or as much as 5 mg/mL IgG. The Protein A ELISA kit contains all necessary reagents, which can be used as supplied (dilution is required for the wash concentrate and Standard Protein A provided). Protein A-free Human IgG is also provided, which can be diluted to match the concentration of test samples containing IgG.

The assay uses a sandwich ELISA format, with pre-coated microtiter wells containing immobilized anti-protein A antibodies that capture protein A in the

sample. The captured protein A is then incubated with biotin-conjugated anti-protein A, forming a "sandwich." A streptavidin-horseradish peroxidase conjugate is then added, which binds to the biotin, and then a chromogenic reagent (TMB) is added, which turns blue in the presence of peroxidase. The blue color development is stopped by adding a stop solution. The resulting yellow color measured at 450 nm is proportional to the amount of captured protein A. The concentration of protein A in the sample is interpolated from a standard curve generated with the Protein A Standard supplied in the kit. Samples containing IgG are interpolated from prepared standards containing IgG. Total assay incubation time is 2.5 hours.

KIT CONTENTS

1. **Microtiter plate 12x8 wells,** coated with anti-Protein A.
2. **Protein A Standard, 20 ng/mL**, 10X, 0.3 mL. Contains preservative (0.1% NaN_3).
3. **Biotinylated anti-Protein A,** 11 mL. Contains preservative: (0.1% NaN_3).
4. **Streptavidin-HRP**, 11 mL. Contains preservative (0.05% Proclin 300).
5. **TMB Substrate Solution**, 15 mL.
6. **Stop Solution**, 15 mL. Contains 2M H_2SO_4. Corrosive (see safety information).
7. **Human IgG,** Protein A-free, 10 mg/mL, 0.5 mL, in phosphate buffer with 0.05% Tween 20 and preservative (0.1% NaN_3). Biohazard - see safety information. Additional IgG is available from Peninsula Laboratories Inc.
8. **20X Wash Buffer Concentrate,** 60 mL. Contains 1% Tween 20. Sufficient for preparation of up to 1.2 L wash solution
9. **Package Insert (instructions for use)**
10. **Plate Map**

Peninsula Laboratories Inc. Protein A ELISA reagents do not contain mercury.

MATERIALS NEEDED BUT NOT PROVIDED

1. Precision pipettes, 10 µL to 1000 µL, with disposable tips
2. Borosilicate test tubes (for boiling IgG samples) to hold 1 mL
3. Distilled or deionized water
4. Graduated cylinders for diluting Wash Buffer
5. Multichannel pipettor (8 or 12 channel)
6. Paper towels
7. Plate sealers or covers
8. Plate shaker
9. Disposable or washable ELISA reagent trays

10. Microtiter plate washer (or wash bottle)
11. Microtiter plate reader with 450 nm filter
12. Graph paper

ADDITIONAL MATERIALS REQUIRED FOR SAMPLES CONTAINING IgG

1. Hot plate and beaker or container (for boiling water bath)
2. Aluminum foil
3. Microcentrifuge tubes, HDPE, to hold 0.5 mL (minimum)
4. Microcentrifuge, to hold microcentrifuge tubes
5. Vortex mixer

REAGENT PREPARATION

All microtiter strips, Biotinylated IgG, Streptavidin-HRP, TMB and Stop Solution are supplied ready to use.

The **20X Wash Buffer Concentrate** requires dilution prior to use. Prepare **1X Wash Buffer** by diluting 50 mL of 20X Wash Buffer Concentrate with 950 mL of distilled or deionized water, to give 1.0 L of 1X Wash Buffer. This same solution is also used as diluent for standards and samples. Save the remaining 20X Wash Buffer Concentrate if needed for treating IgG samples prior to assay (see below).

Standard Protein A is supplied at 20 ng/mL (10X). This allows Protein A to be spiked into the IgG provided at 1/10 volume to prepare Protein A standards in the presence of IgG. See "Standard Curve Preparation" below.

The **Human IgG** provided must first be diluted to the same concentration as the samples (which must be ≤5 mg/mL) before Protein A is added. Use 1X Wash Buffer to make the appropriate dilution of the Human IgG supplied in the kit.

STANDARD PROTEIN A FOR SAMPLES CONTAINING IgG

1. Dilute the Human IgG provided in the kit with 1X Wash Buffer to a concentration equal to that of the eluted samples and ≤ 5 mg/mL. Final volume of diluted IgG needed is 0.45 mL. Save stock IgG for future assays.
2. Add 50 µL of 10X Protein A Standard to 0.45 mL of IgG solution from Step 1 and vortex. Final Protein A concentration is 2000 pg/mL. IMPORTANT: Let stand for 30 ± 5 minutes at room temperature (22 ± 5 °C) before further processing.
3. Process the Protein A - IgG standard along with the IgG samples as described above under "Treatment of IgG Samples" below.
4. After boiling and centrifuging, take the supernatant and follow the instructions below to "Prepare Standard Dilutions."

TREATMENT OF IgG SAMPLES (INCLUDING PROTEIN A COLUMN ELUATES)

Neutralize column eluates and determine the IgG concentration of the samples by standard laboratory procedures. Adjust all sample volumes with neutral buffer to make all IgG concentrations equivalent and ≤ 5 mg/mL. Record the dilution factor for correction of the final value.

If Tween 20 is not already in the neutralization buffer, add a 1/20 volume of 20X Wash Buffer Concentrate to each sample to be assayed. Final Tween 20 concentration will be 0.05%.

Only use glass tubes. Place at least 0.3 mL of neutralized IgG sample in a small test tube and cover the opening with aluminum foil, a marble or other loose cap. Place tubes in a boiling water bath for 5 ± 1 minutes. Allow to cool for at least 5 minutes at room temperature. Transfer the entire contents to plastic microcentrifuge tubes and clarify by centrifugation for 4 minutes at 8,000 rcf or higher (for example, 13,000 rpm on any Eppendorf model microcentrifuge). Use supernatant for analysis.

Boiling and centrifuging are not necessary for samples that do not contain IgG.

STANDARD CURVE PREPARATION FOR SAMPLES WITHOUT IgG

1. Prepare a 2000 pg/mL Protein A standard by adding 50 µL of the 10X Protein A Standard to 450 µL of 1X Wash Buffer, giving a final Protein A concentration of 2000 pg/mL.
2. Follow the instructions below to "Prepare Standard Dilutions."

PREPARE STANDARD DILUTIONS

The standard curve can be prepared directly in the ELISA plate using twofold serial dilutions.

1. Place 100 µL of 1X Wash Buffer in wells B1 - H1 and (for duplicate analysis) B2 - H2.
2. Add 100 µL of the 2000 pg/mL Protein A standard (with or without IgG) to wells A1 and A2.
3. Add 100 µL of the 2000 pg/mL Protein A standard (with or without IgG) to wells B1 and B2.
4. Mix the contents of wells B1 and B2 with a pipettor (using 2 channels of a multichannel pipettor set at 100 µL) by aspirating and expelling the solution 5 times. Be careful to keep the tips submerged and avoid foaming. Do not scrape the interior of the wells with the pipette tips.
5. Aspirate 100 µL and transfer to wells C1 and C2.
6. Continue mixing and transferring through wells G1 and G2.
7. Withdraw 100 µL from wells G1 and G2 and discard.
8. Wells H1 and H2 contain diluent only for 0 pg/mL.

Alternatively, standards can be prepared in glass test tubes at the desired concentrations (between 50 and 2000 pg/mL) and added to pre-defined wells in the assay plate. The assay should provide a linear response ($R^2 > 0.95$) for Protein A concentrations between 50 – 2000 pg/mL.

ASSAY PROCEDURE

MAKE SURE THAT ALL REAGENTS ARE AT ROOM TEMPERATURE (22 ± 5 °C) BEFORE PROCEEDING. Duplicate analysis is recommended.

Remove any strips not needed for the assay from the frame and store them in the foil pouch. (After the assay, keep the plate frame for future use with the stored strips.) Duplicate analysis is recommended. It is good practice to invert the plate and tap it firmly on a paper towel after each wash to remove residual wash solution.

1. Follow the instructions above to prepare standard dilutions directly in the plate. This will yield 2000, 1000, 500, 250, 125, 62.5, 31.3 and 0 pg/mL standards into wells A1 and A2 through H1 and H2, respectively. If standards were prepared in tubes, add 100 µL of each standard to the appropriate wells.
2. Add 100 µL of each sample into successive pairs of wells, and record their position on the plate map.

3. Cover the plate and incubate at room temperature on a shaker set at 200 ± 20 rpm for 60 ± 2 minutes.
4. Wash each well 4 times with 1X Wash Buffer; fill and empty the wells each time.
5. Add 100 µL/well of Biotinylated Anti-Protein A into the wells.
6. Cover the plate and incubate at room temperature on a shaker set at 200 ± 20 rpm for 60 ± 2 minutes.
7. Wash each well 4 times with 1X Wash Buffer.
8. Add 100 µL/well of Streptavidin-HRP into each well.
9. Cover the plate and incubate at room temperature on a shaker set at 200 ± 20 rpm for 30 ± 1 minutes.
10. Wash each well 4 times with 1X Wash Buffer.
11. Add 100 uL/well of TMB Substrate Solution.
12. Incubate at room temperature on a shaker set at 200 ± 20 rpm for 10 ± 0.5 minutes.
13. Add 100 uL/well of Stop Solution.
14. Measure the absorbance at 450 nm of each well using a microtiter plate reader. Blank the instrument on air. For dual wavelength reader, use a 630 or 650 nm reference wavelength. The color in the plate is stable for up to 6 hours at room temperature (22 ± 5 °C).

ASSAY VALIDITY

- The 0 pg/mL absorbance readings should be less than 0.25 OD.
- The difference between the mean absorbances of the 125 pg/mL and the mean 0 pg/mL standards should be at least 0.10 OD in the absence of IgG.
- The mean absorbance for the 2000 pg/mL Standard should be greater than 1.5 OD in the absence of IgG.
- A linear regression analysis of the standards should produce an R^2 value > 0.95.
- Deviation from the assay procedure may lead to unreliable results.
- Mixing reagents from different lots may lead to unreliable results.

CALCULATION OF RESULTS

Calculate the mean absorbance for each of the duplicate wells. Plot the standard curve on graph paper with the Protein A concentration (pg/mL) on the X axis and the mean absorbance on the Y axis. From the absorbance of the samples, find the corresponding Protein A concentration on the X axis. If the samples were diluted, correct the result for dilution factor.

Alternately, concentration values may be calculated from a linear regression of the standard curve, provided that the R^2 value is > 0.95. The equation of the line is:

$$y = mx + b$$

where y = mean absorbance,
m= slope,
x = concentration (pg/mL),
b = y intercept.

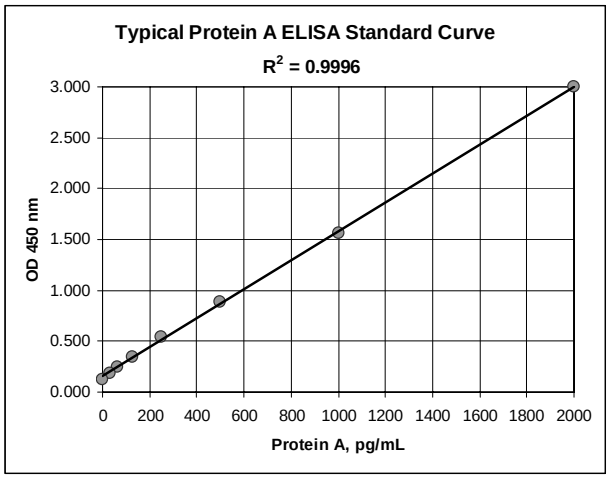
To find the concentration, solve for x:

$$x = (y-b) / m$$

TYPICAL RESULTS

The results shown below are for illustration purposes only. **DO NOT** use these results to calculate results from an assay.

Standard pg/mL	Mean OD 450 nm
2000	2.998
1000	1.561
500	0.917
250	0.542
125	0.344



62.5	0.252
31.25	0.189
0	0.125

Sample	Mean OD	Protein A, pg/mL
Unknown 1	1.11	686
Unknown 2	0.223	47

Recovery of Protein A from Samples Containing IgG

The following table illustrates the recovery of Protein A from samples containing human IgG present at concentrations of 0 mg/mL, 1 mg/mL and 5 mg/mL. Protein A concentrations were calculated using Protein A standards prepared in solutions containing the same IgG levels as the samples.

Protein A Added	0 mg/mL IgG		1 mg/mL IgG		5 mg/mL gG	
	Recovered	%	Recovered	%	Recovered	%
pg/mL	pg/mL	%	pg/mL	%	pg/mL	%
50	52	105%	52	104%	54	109%
1600	1706	107%	1692	106%	1518	95%

REFERENCES

1. Forsgren,A., Ghetie,V., Lindmark. R., Sjöquist,J., Protein A and its exploitation. In: *Staphylococci and Staphylococcal infections 2* (Eds. C.S.F. Easmon and C. Adlam), pp. 429-480, Academic Press Inc., London 1983.
2. Lindmark,R., Thoren-Tolling,K., Sjöquist,,J., (1983) Binding of immunoglobulins to Protein A and immunoglobulin levels in mammalian sera. *J. Immunol. Methods*, 62, 1-13.

NOTES AND WARNINGS

- **Handling of pipettes**: Since incubation volumes are small, careful pipetting is crucial to the success of the assay. Poor technique may cause droplets to stick to the upper wall of the wells and spray (aerosols) may contaminate adjacent wells causing variation in results.
- **Handling the immunoplate**: Do not touch the bottom of the plate. This leaves grease, dirt, etc. that will adversely affect the optical absorbance reading.
- **Water purity**: The performance of the kit is sensitive to contaminants in water. If possible, use sterile deionized water or USP pure water (18MOhm). When drawing water from a water purification system, we recommend purging the system before using the water to eliminate any contaminants, which may have built up in the water lines.
- **Storage**: Store the kit in a cool (2-8°C) dry area upon receipt. Since all incubations and procedures are performed at room temperature, the reagents, immunoplate, and samples should be equilibrated to room temperature before use. Do not open reagents and immunoplate while they are cold.

SAFETY INFORMATION

The physical and chemical properties of the reagents contained in this kit have been tested individually.
It is strongly recommended that laboratory employees wear lab coats, gloves, and safety glasses when handling the reagents provided with this kit.

Hazardous Ingredients

- **Human IgG:**
  - US: Biohazard. Handle as if capable of transmitting infectious agents.
  - EU: Biohazard "B".
- **Sodium azide (NaN_3):** may react with lead and copper plumbing to form highly explosive metal azides.
- **Sulfuric acid (H_2SO_4): Danger! Harmful if inhaled. Corrosive.** Hygroscopic. May cause irritation and burns of eyes, skin, digestive and respiratory tract.

Physical and Chemical Data

Components are stable in closed containers under normal temperatures and pressures. No hazardous polymerization known. Peninsula Laboratories Inc. Protein A ELISA reagents do not contain mercury.

Fire and Explosion Data

Components are non-combustible with negligible fire hazard when exposed to heat or flame. Fire fighting media should be appropriate to burning material. Sodium azide in human IgG may react with lead and copper plumbing to form highly explosive metal azides.

Health Hazards

Components may be harmful by inhalation, ingestion, or skin absorption and may cause skin irritation or eye irritation.

- In case of eye contact, flush eye with water and contact a physician.
- In case of skin contact, wash skin with soap and water.
- In case of inhalation, remove from exposure to fresh air immediately; if breathing is difficult, give oxygen; get medical aid immediately.
- In case of ingestion, do NOT induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.

Reactivity Data

Components are stable in closed containers under normal temperatures and pressures.

Spill and Disposal Procedures

For spills, ventilate area and wash spill site. For disposal, please dispose in accordance with local regulation.

Handling and Storage Information

Safety glasses, gloves, and a full-length lab coat should be worn to prevent unnecessary contact.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. Peninsula Laboratories Inc. shall not be held liable for any damage resulting from the handling or use of the above product.

ORDERING INFORMATION

For additional kits or our most current catalog products, please visit our web site at www.bachem.com or call us at:

Tel: (800) 922-1516 (for U.S. and Canada)
(650) 592-5392 (for International)
Fax: (650) 595-4071

E-mail: immunosales@usbachem.com

TECHNICAL SUPPORT

If you need technical information or assistance with assay procedures, please call:
Technical Service Department at

Tel: (800) 922-1516 (for U.S and Canada)
(650) 592-5392.(for International)

E-mail: immunology@usbachem.com

Our staff will be happy to answer your questions about this or any other Peninsula Laboratories Inc. product.

GUARANTEE AND LIMITATION OF REMEDY

Peninsula Laboratories Inc., makes no guarantee of any kind, expressed or implied, which extends beyond the description of the material in this kit, except that these materials and this kit will meet our specifications at the time of delivery. Customer's remedy and Peninsula Laboratories Inc.'s sole liability hereunder is limited to either refunding the purchase price or replacing the material(s) that does not meet our specifications. By the acceptance of our products, the customer indemnifies and holds Peninsula Laboratories Inc. harmless against, and assumes all liability for the consequences of the use or misuse of products by the customer, its employees, or others.