ab133036 – Protein A ELISA Kit

Instructions for Use

For quantitative detection of natural and recombinant Protein A in neutralized buffers.

This product is for research use only and is not intended for diagnostic use.

Version 6b Last Updated 5 October 2020

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1. BACKGROUND

Abcam's Protein A *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of natural and recombinant Protein A in neutralized buffers.

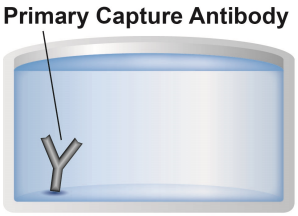
A chicken IgY antibody specific to Protein A has been precoated onto 96-well plates. Standards or test samples are added to the wells, incubated and then washed. A Protein A polyclonal antibody is then added, incubated and washed. An HRP conjugated antibody is then added incubated. The plate is washed once more and the TMB substrate is then added which HRP catalyzes, generating a blue coloration after incubation. A stop solution is added which generates conversion to yellow color read at 450 nm which is proportional to the amount of analyte bound.

Staphylococcus aureus Protein A is a cell wall constituent that is characterized by its binding affinity to the Fc portion of some immunoglobulins, especially the IgG class. Protein A is a 42 kilodalton protein that has four repetitive domains rich in aspartic and glutamic acids but devoid of cysteine. The IgG binding domain (domain B) consists of three antiparallel alpha-helices, the third of which is disrupted when the protein is complexed with Fc2. Protein A participatesin a number of different protective biological functions including anti-tumor, toxic and carcinogenic activities. There are antifungal and antiparasitic properties in addition to its ability to act as an immunomodulator. Staphylococcal Protein A (with other surface proteins) is able to induce a Th1 type of response by eliciting the production of cytokines such as IFNγ, TNF-α, IL-1α, IL-1β, IL-2 and IL-4. Protein A is used during the microscopic in situ visualization of biologically important molecules and to purify antisera. Extracorporeal therapeutic immunoadsorption techniques utilize Protein A in the treatment of proteinuria in nephrotic syndrome and severe autoimmune diseases such as rheumatoid arthritis, coeliac disease, and systemic lupus erythematosis. Protein A from the Cowan I strain of

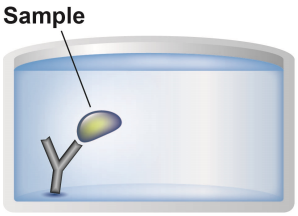
INTRODUCTION

Staphylococcus aureus has therapeutic and prophylactic applications in the control of Leishmania infections in animals. The anti-leishmanial effects may be mediated through the activation of macrophages resulting in enhanced phagocytosis of the parasites. Protein A induced TNF-α and IL-2 is associated with the control of splenic cell apoptosis in mice.

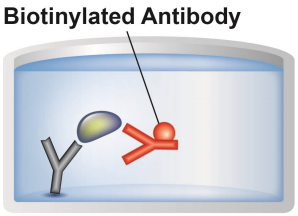
2. ASSAY SUMMARY



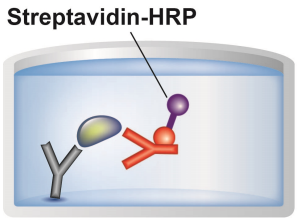
Prepare all reagents, samples and standards as instructed.



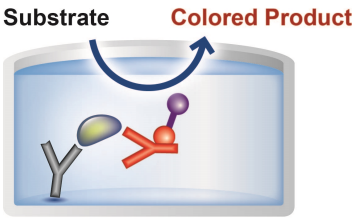
Add standard or sample to each well used. Incubate at room temperature



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Streptavidin HRP solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read at 450nm immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results
- Care should be taken handling the Standard and Conjugate material because of the known and unknown effects of Protein A

4. STORAGE AND STABILITY

Store all components at 4°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Chicken anti-Protein A Microplate (12 x 8 wells)	96 Wells	4ºC
Protein A HRP-Streptavidin Conjugate	10 mL	4ºC
Protein A Antibody	10 mL	4ºC
Protein A Standard	1 vial	4ºC
Assay Buffer 13	55 mL	4ºC
20X Wash Buffer Concentrate	100 mL	4ºC
TMB Substrate	10 mL	4ºC
Stop Solution 2	10 mL	4ºC
Plate Sealer	2 Units	4ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader capable of reading at 450 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- 2 M hydrochloric acid
- Deionized water
- Ethanol
- Hexane
- Ethyl acetate

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 **Protein A Conjugate**

Allow the Protein A HRP-Streptavidin Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 **1X Wash Buffer**

Prepare the 1X Wash Buffer by diluting 50 mL of the Wash Buffer Concentrate in 950 mL of deionized water. This can be stored at room temperature until the kit's expiration date, or for 3 months, whichever comes first. Mix thoroughly and gently.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 30 minutes of preparation. If samples contain IgG, then standards must be boiled. See section 11 for instructions.

10.1 Allow the 10,000 pg/mL Protein A Standard solution to equilibrate to room temperature. The standard solution should be aliquoted and stored at -20°. Avoid repeated freeze-thaw cycles.

10.2 Label eight tubes with numbers 1 – 8.

10.3 Add 500 µL of Assay Buffer 13 into tubes numbers 2 – 8.

10.4 Prepare a 1,000 pg/mL **Standard 1** by adding 100 µL of the 10,000 pg/mL Stock Standard to 900 µL of the Assay Buffer 13 into tube 1. Mix thoroughly and gently.

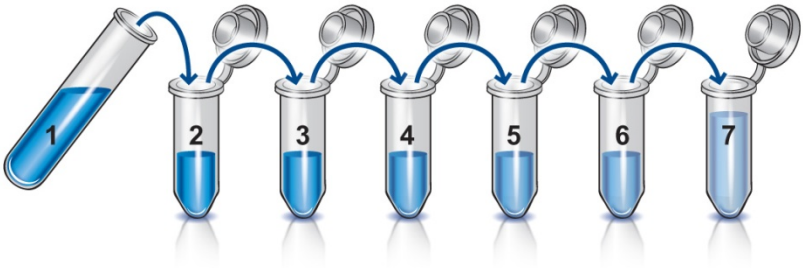
10.5 Prepare **Standard 2** by transferring 500 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 500 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes 4 through to 7.

10.8 **Standard 8** contains no protein and is the **blank control**.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	100	900	10,000	1,000
2	Standard 1	500	500	1,000	500
3	Standard 2	500	500	500	250
4	Standard 3	500	500	250	125
5	Standard 4	500	500	125	62.5
6	Standard 5	500	500	62.5	31.3
7	Standard 6	500	500	31.3	15.6
8	None	-	500	-	0



11. SAMPLE COLLECTION AND STORAGE

The Protein A ELISA kit is compatible with natural and recombinant Protein A samples in neutralized buffers. Samples containing antibodies must be prepared in the following manner prior to running the assay.

11.1 Determine the concentration of antibody present in the eluted samples. Dilute all samples to 1 mg/mL with Assay Buffer 13.

11.2 Prepare standards as described in section 10.

11.3 Aliquot a minimum of 500 µL of each sample and standard into a microcentrifuge tube with a hole in the lid. This volume will allow for duplicates of each sample and standard to be measured in the assay.

11.4 Incubate samples, standard samples for 5 minutes in a boiling water bath.

11.5 Allow samples to cool for 5-7minutes at room temperature. Centrifuge samples for four minutes at 13,800 x g at room temperature.

11.6 Use supernatants from the cooled sample and standard tubes directly in the assay.

Depending on the species and type of antibody present in the sample it may be necessary to modify the above protocol. For example, samples containing Human IgG, an antibody with high affinity for Protein A, require dilution to 50 µg/mL hIgG and an additional 15 minutes of boiling to achieve accurate Protein A concentrations.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 100 µL of standards and samples into the appropriate wells.

13.3 Incubate the plate at room temperature on a plate shaker for 1 hour at ~500 rpm. The plate may be covered with the plate sealer provided.

13.4 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 Washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.5 Add 100 µL of Protein A antibody into each well.

13.6 Seal the plate. Incubate at room temperature on a plate shaker (~ 500 rpm) for 1 hour.

13.7 Wash as described above in step 13.4.

13.8 Add 100 µL of Protein A HRP-Streptavidin Conjugate into each well.

13.9 Seal the plate. Incubate at room temperature on a plate shaker (~ 500 rpm) for 30 minutes.

13.10 Wash as described above in step 13.4.

13.11 Add 100 µL of the Substrate solution to every well. Incubate at room temperature for 15 minutes on a plate shaker at ~500 rpm.

13.12 Add 100 µL Stop Solution into each well.

13.13 Immediately read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm.

14. CALCULATIONS

A four-parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

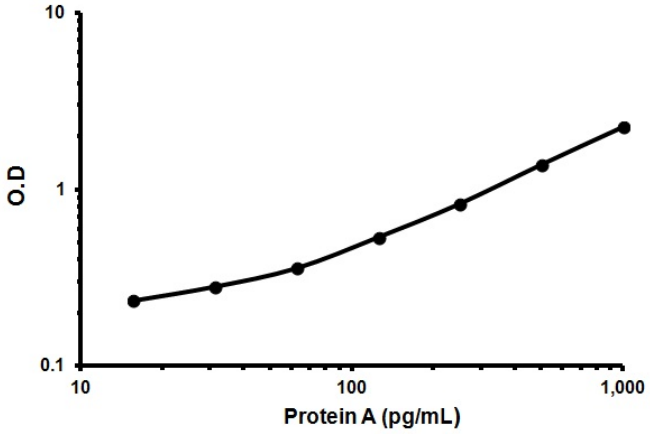
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the blank OD from the average OD bound:

  Average Net OD = Average Bound OD - Average Blank OD

- Plot the average Net OD for each standard versus Concentration of Protein A for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Net OD	Protein A (pg/mL)
Standard 1	2.243	1,000
Standard 2	1.374	500
Standard 3	0.825	250
Standard 4	0.533	125
Standard 5	0.356	62.5
Standard 6	0.280	31.3
Standard 7	0.233	15.6
Standard 8	0.202	0
Unknown 1	0.324	48.2
Unknown 2	1.123	379.9

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

Sensitivity was calculated as the ratio of the mean OD plus 2 standard deviations of 16 replicates of the 0 pg/mL standard to the mean of 16 replicates of the lowest standard, multiplied by the concentration of that standard (15.62 pg/mL). This value was determined to be 9.01 pg/mL.

LINEARITY OF DILUTION –

Protein A was spiked into acidic 0.1 M Citric Acid and 0.1 M Glycine to model samples eluted off Protein A columns. These samples were neutralized with a 2 mM Tris buffer and then serially diluted 1:2 in the kit assay buffer. The Citirc acid returned a Recovery of 99%, and the Glycine recovery was 95%.

PRECISION –

	Protein A (pg/mL)	Intra-Assay %CV
Low	127.9	5.2
Medium	364.3	6.4
High	727.8	5.5

	Protein A (pg/mL)	Inter-Assay %CV
Low	103.6	13.4
Medium	209.7	8.1
High	378.3	8.0

17. ASSAY SPECIFICITY

The Protein A ELISA Kit recognizes natural and recombinant forms of Protein A. No detectable cross-reactivity with other relevant proteins.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. NOTES

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)