Protein A (PA) ELISA Kit

(Non-Native Protein)

48 Well

Catalogue Number: NEGEP0890-48T

Valid Period: 6 months

For samples:

ELISA Kit for the quantitative Measurement of Protein A (Non-Native Protein) Residues in Protein Purification Process, and End-Product (purified fermentation broth, cell culture supernatant, etc.)

FOR RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. APPLICATION

The Protein A ELISA kit is applicable in the field of biotechnology and biomedicine, it is for quantitative measurement of Protein A residues in the purification process of biopharmaceutical production, for examples like purified fermentation broth and cell culture supernatant, etc. This ELISA kit is for research use only, it should not be used in clinical diagnostic procedures.

2. BACKGROUND

Staphylococcus Aureus Protein A (SPA or Protein A) is a 42kDa surface protein named MSCRAMM(Microbial Surface Components Recognizing Adhesive Matrix Molecules), it is originally found in the cell wall of the bacteria Staphylococcus aureus. It is composed of five homologous Ig-binding domains that fold into a three-helix bundle. Each domain is able to bind proteins from many mammalian species, most notably IgGs. Protein A can bind with strong affinity to human IgG1, human IgG2, mouse IgG2a and IgG2b, and with medium affinity to human IgM, IgA and IgE as well as mouse IgG3 and IgG1. Protein A can not bind to human IgG3,IgD or react with human IgM, IgA, IgE..

Protein A is widely used in biomedicine field because of its strong affinity to immunoglobulins. Protein A used to produce antibodies in the medicine field are mostly the ones binding to stationary-phase Chromatographic Resin.

In affinity chromatography, even though Protein A is covalently attached to the agarose matrix, a small amount can still detach from the column and enter the elution pool. Leakage of protein A may contaminate the antibodies during the production process. Protein A has the ability to interact with multiple hosts, which indicate it might be a virulence factor in Staphylococcus aureus infection. Currently, the Protein A used for antibody purification in the market mainly consists of naturally structured and recombinantly expressed products from E. coli, which share a basic structural similarity. Besides these, there exists another type of Protein A with a non-natural source and significantly distinct structure from the former two categories, such as GE's MabSelect SuReTM series. Due to the unique structure of this Protein A variant, some antibodies can bind to it to varying degrees. Conventional purification methods fail to completely separate them, leading to inaccurate quantification.

To ensure the quality of antibodies purified by Protein A affinity chromatography, we have successfully developed Protein A ELISA kit.

The kit employs denaturation to completely separate antibody products bound to non-natural Protein A, eliminating antibody interference during the detection process. This enables detection of impurities below 100 pg/ml with simplicity of operation and high sensitivity, ensuring accurate test results. The kit is also applicable for the quantitative measurement of protein A in the samples with highly concentrated antibody such as purified antibody end products.

3. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the double antibody sandwich Enzyme Linked Immunosorbent Assay to detect the concentration of protein A in samples. Before starting the ELISA experiment, mix the samples and serially diluted standards with denaturing solution, and incubate at room temperature to completely dissociate Protein A from the antibodies. After pretreatment, add the detection antibody to the microplate wells pre-coated with chicken anti-Protein A antibody. Then add the denatured standards and samples. Incubate at room temperature to allow the specific binding of antigen-antibody, capturing Protein A from the samples and standards onto the microplate. After incubation, a sandwich complex forms with the coating antibody, Protein A, and detection antibody. Wash the plate to remove unbound substances. Then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP, and finally turns to yellow immediately after adding the stop solution. Color of TMB substrate positively correlated with total protein A bound in the initial steps. The color is measured by spectrophotometrically with wavelength of 450nm. The concentration of protein A in samples is then determined by comparing the O.D. of the samples to the standard curve.

4. KITS' ADVANCEMENT

1) High Specificity: Capture antibody and detection antibody respectively identify different epitopes of the antigen, which maximizes the specificity of the reaction.
2) High Stability: The assay employs coating antibodies that recognize non-native Protein A, specific broad-spectrum protein stabilizers, and a microplate treatment process to enhance thermal stability and result reproducibility.

3) Optimal Diluent Buffer: Use a specific buffer optimized for human serum samples to eliminate matrix interference, suitable for quantifying cytokines in serum, plasma, and other conventional samples.

4) Short Experiment Time: The optimized process takes only 1.5 hours, significantly reducing the time compared to traditional boiling methods.

5. MATERIALS AND EQUIPMENTS (Note: Storage at 2-8℃)

	REAGENTS	SPECIFICATION	QUANTITY
1	Pre-Coated Microplate (Detachable)	48 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -2ug/ml	20ul	1 vial
3	HRP-conjugated antibody(400×)	25ul	1 vial
4	Sample Pretreatment Solution	10ml	1 vial
5	TMB Substrates	10ml	1 vial (Avoid Light)
6	Stop Solution	10ml	1 vial
7	Wash Solution (100×)	10ml	1 vial
8	Diluent Buffer (10×)	10ml	1 vial
9	Plate Sealer		4 pieces
10	Instruction Manual		1

6. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000 μl pipettor and disposable sterilized tips
2) Multichannel pipettor
3) 1L sterilized deionized water or ultrapure water
4) Sterilized EP tubes
5) Absorbent Paper
6) Microplate reader
7) High-speed centrifuge
8) Microplate washer or washing bottles

9) Incubator or water bath (Optional)

10) Data analysis and graphing software

11) Preparation of PBS: NaH2PO4 0.2g, Na2HPO4·12H2O 2.9g, NaCl 8g，KCl 0.2g. Then dilute these to 1000ml with distilled water

7. SPECIMEN COLLECTION AND STORAGE

Serum - Use a serum separator tube and allow samples to clot for 1-2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 1000×g (or 3000 rpm) for 15 minutes. Collect serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed 300-500mg before homogenization. Minced the tissues to small pieces and homogenized them in 500ul of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500×g (or 5000 rpm).Collect the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.

Cell lysates - Cells should be lysed according to the following directions.

1) Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
2) Wash cells three times in PBS. As for the collection of the samples, the amount of cells should be no less than 10^8 in 200ul PBS.
3) Cells were resuspended in PBS and subjected to ultrasonication for 3 times. Alternatively, freeze cells at -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.
4) Centrifuge at 1000×g (or 3000 rpm) for 15 minutes at 2-8°C to remove cellular debris.

Assay immediately or store samples at -20°C or -80°C.

Cell Culture Supernatant -Collect cultured cell, centrifuge them at 1000×g (or 3000 rpm) for 15 minutes, and collect the supernatant for immediate assay or store samples in aliquots at -20°C or -80°C.

NOTE:

- Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles.
- Some chemical solvents may interfere with this experiment, such as SDS, Triton, etc.
- The precipitates in the sample solution can interfere with ELISA. Make sure to centrifuge and remove them.
- Do not use lipemic or hemolyzed samples, as they can interfere with ELISA and produce inaccurate results.
- Do not use heat-treated specimens.

8. PREPARATION BEFORE ASSAY

1) Please read through this manual carefully before using.The experiment can be conducted at room temperature (20-25℃). If the temperature is higher or lower, the kit can be placed in a 37°C incubator.
2) The frozen samples should be slowly restored to room temperature and then mix well, centrifuge the samples at 12000 rpm for 1 minute. Collect the supernatant for immediate assay.

NOTE:

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Influenced by the factors including low cell viability, small cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples, and lead to inaccurate results.
- The optimal pH value of the sample is between 7.0-7.4.

9. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30mins before using.

2) The preparation of Wash Solution (1×): Dilute 10 mL of Wash Solution (100×) with 990 mL of deionized water or ultrapure water to prepare 1000 mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

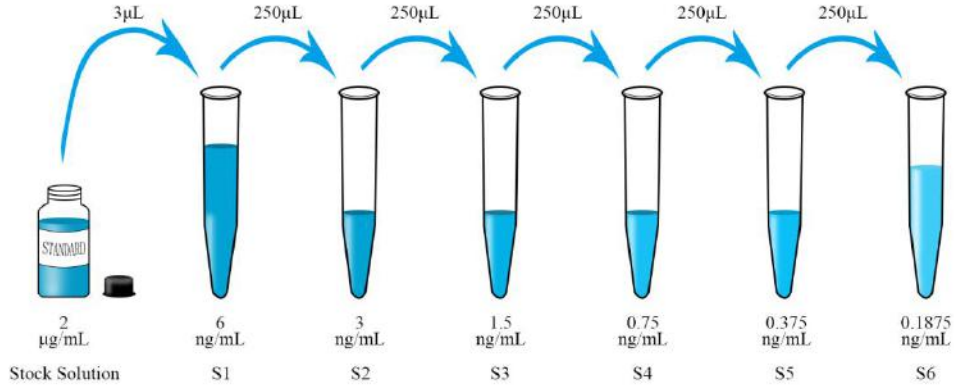
3) The preparation of Diluent Buffer: Dilute 10 mL of Diluent Buffer (10×) with 90 mL of deionized water or ultrapure water to prepare 100 mL of Diluent Buffer (1×) . If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

4) Sample Pre-dilution: If the predicted concentration of your sample exceeds the upper detection limit of this kit, it is recommended to dilute the sample with PBS

5) Prepare Standards: When preparing the standards, label 6 tubes, S1-S6 and add a certain volume of diluent:S1 (997ul), S2 to S6 (each 250ul).

   Add 3ul of the Standard (Stock Solution-2ug/ml) into the tube labeled as S1. Shake well and then pipette 250ul Standard solution (6ng/ml) from S1 to S2. And then produce a 2-fold dilution series until S6 .

   NOTE: There are 6 points of diluted standards, S1: 6ng/ml, S2: 3ng/ml, S3: 1.5ng/ml, S4: 0.75ng/ml, S5: 0.375ng/ml, S6: 0.1875ng/ml.



6) Sample Preparation: Mix pre-diluted test samples and standards with sample pretreatment solution provided in the kit at a 2:1 volume ratio(e.g., add 50 µL pretreatment solution to 100 µL standard or sample). Gently tap the mixture 10-15 times, and incubate at room temperature for 5-10 minutes before use.

7) Prepare Detection Antibody (1×)：Take 12.5 µL of HRP-conjugated antibody (400×) and add it to 5 mL of Diluent Buffer (1×). Mix well.

10. ASSAY PROCEDURES

1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4° C. (Note: The microplate frame can be reused).

2) Add 100ul of Detection Antibody (1×) to each well, then add 25 µL of the Standards and samples. Cover the plate with a sealer and incubate at room temperature for 1 hour at 400-600 rpm.

3) Automated Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no no moisture appears.
- Fill each well with 300ul Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times. Note: There should be no moisture appears in the fifth washing step.

4) Add 100ul TMB substrate to each well. Cover the plate with a new sealer, incubate at room temperature for 10 to 30 mins. (Avoid sunlight).

5) Add 50ul of Stop Solution to each well to stop the reaction, mix well. Immediately run the microplate reader and conduct measurement at 450nm.

11. NOTES

1) **Sample Preparation**

a) After collection, the samples should be aliquoted and stored at -20℃ (less than 3 months) or -80 ℃ (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8° C.

b) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.

c) If turbidity appears in the stock solution (Standard, Detection antibody and HRP-Streptavidin), mix gently or pipette up and down for several times.

d) It is recommended that all standards, controls and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

e) Do not use hyperlipidemia or hemolysis samples, which may interfere with ELISA and lead to inaccurate results.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

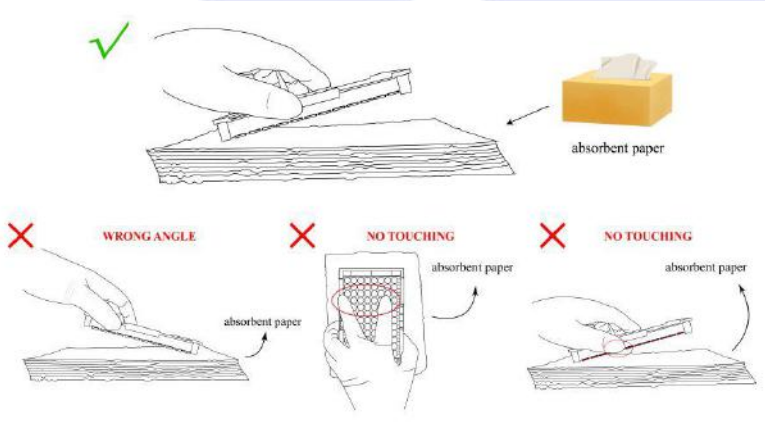
e) TMB is easily contaminated. please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

i) Schematic diagram of tapping the plate:



12. CALCULATION OF RESULTS

1) Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.

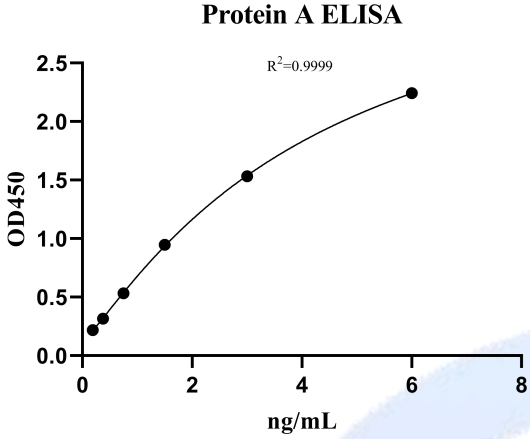
2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.

3) Calculate the concentration of samples corresponding to the mean absorbance from the standard

curve.

4) Standard curve for demonstration only.

APPENDIX 1 : EXAMPLE OF STANDARD CURVE



ng/ml	OD450
6	2.242
3	1.532
1.5	0.946
0.75	0.533
0.375	0.315
0.1875	0.217

13. QUALITY CONTROL

1) Sensitivity:

   LOD: 47pg/ml

   LOQ: 187pg/ml

2) Intra Variation%: 7-8

3) Inter Variation%: 6-9

4) Recovery Rate:

   In the first experiments, the matrix effect on experimental results should be confirmed through spike recovery. It is recommended to add one volume of standard solution (6 ng/mL) to every three volumes of the sample. Calculate the recovery rate using the formula: (actual concentration after spiking - concentration before spiking) / theoretical concentration (3 ng/mL). A recovery rate of 80-120% is considered acceptable.

   Matrix sources that interfere with sample detection include extreme pH (pH < 5 or pH > 8.5), high salt concentrations, and detergents, which can lead to low recovery rates. High concentrations of antibody products may also affect results; therefore, it is advisable to dilute the sample multiple times before the experiment.

5) Hook Capacity: This ELISA kit is applied to the two-step sandwich Enzyme Linked Immunosorbent Assay. Samples with high concentrated protein A will be effected by Hook Capacity. Please dilute the samples with the diluent provided till it's within the detection range of the ELISA kit.

8) Limitation: This ELISA kit is not suitable for the samples containing NaN3. NaN3 is a strong inhibitor for HRP that will decrease the concentration of the target protein.

14. SAFETY NOTES

1) This kit contains small amount of 3, 3', 5, 5'-Tetramethylbenzidine (TMB) in Substrate B. TMB is non-carcinogenic but it is hazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse affected area with plenty of water.

2) The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, and face protection.

15. CONTACT US

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