# Protein A (PA) ELISA Kit

**48 Well**

**Catalogue Number: NEGES0890-48T**

**Valid Period: 6 months**

**For samples:**

**ELISA Kit for the quantitative Measurement of Protein A 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!**

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## 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.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.

A small amount of protein A used in affinity chromatography may fall off the chromatographic column, and enter into elution tank. 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. To ensure the quality of antibodies purified by Protein A affinity chromatography, we have successfully developed Protein A ELISA kit. 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. The microtiter plate has been pre-coated with chicken anti-protein A antibody , standards or samples are then added to the microtiter plate wells

and protein A if present, will bind to the antibody pre-coated wells under specific conditions. After washing, then a Horseradish Peroxidase(HRP)-conjugated detection rabbit anti-protein A antibody is added to the wells, under 37℃ incubation, to form a precoated antibody-protein A-detection antibody sandwich complex. 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 experiment uses high-quality coated antibodies and antigens, and also applies the broad-spectrum protein stabilizers, and antibody pre-coated microplate treatment to increase the thermostability of the microplate, and reproducibility of results.

## 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 | 10ul | 1 vial |
| 3 | HRP-conjugated antibody(200×) | 40ul | 1 vial |
| 4 | TMB Substrates | 10ml | 1 vial (Avoid Light) |
| 5 | Stop Solution | 10ml | 1 vial |
| 6 | Wash Solution (100×) | 10ml | 1 vial |
| 7 | Diluent Buffer (10×) | 10ml | 1 vial |
| 8 | Diluent Ingredient | 0.5g | 1 tube |
| 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 (37℃/80℃)
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

**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 heat-treated specimens.

## 8. PREPARATION BEFORE ASSAY

1) Please read through this manual carefully before using, and set the temperature at 37 °C for Incubator or Water bath.
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.
3) Sample Dilution: Please predict the concentration before assaying. If the predicted concentration of your sample exceeds the maximum detection limit of this kit, it is recommended to dilute the sample with PBS.

NOTE:

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- 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.

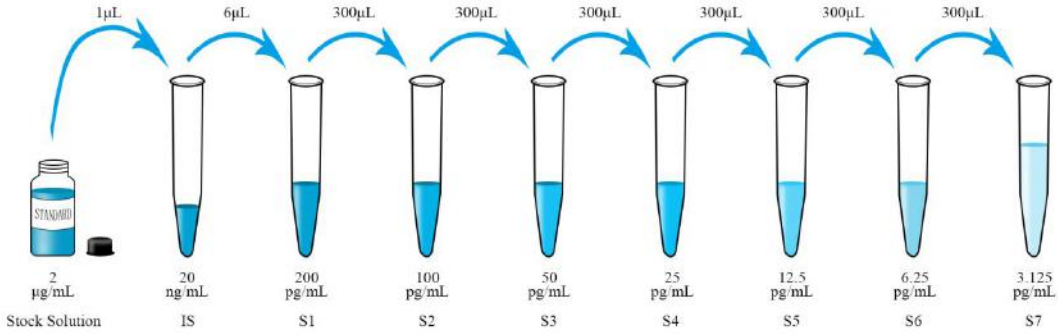
## 9. REAGENT PREPARATION

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. Then add 0.5g Diluent Ingredient into 100ml Diluent Buffer (1×), mix well as the final Diluent Buffer for standards, samples and detection antibodies.
4) The preparation of the samples: Once the water bath reaches and stabilizes at 80°C, bathe the samples for 2 minutes. Then take out the samples, after the samples returning to room temperature, mix evenly, centrifuge at 12,000 rpm for 1 minute. Collect the supernatant as the samples to be tested.

5) Prepare Standards: When preparing the standards, label 8 tubes, IS, S1-S7 and add a certain volume of diluent: IS(99ul),S1 (594ul), S2 to S7 (each 300ul).

Add 1ul of the Standard (Stock Solution-2ug/ml) into the tube labeled as IS（20ng/ml），then take 6ul IS solution to the tube labeled as S1. Shake well and then pipette 300ul Standard solution (200pg/ml) from S1 to S2. And then produce a 2-fold dilution series until S7.

NOTE: There are 7 points of diluted standards, S1: 200pg/ml, S2: 100pg/ml, S3: 50pg/ml, S4: 25pg/ml, S5: 12.5pg/ml, S6: 6.25pg/ml, S7: 3.125pg/ml.




6) Extract 25ul of HRP-conjugated antibody(200×), and add it into 5ml Diluent Buffer (1×) to reach its working concentration (1×) and mix gently

## 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) Secure the desired plate in the holder then add 100ul of diluted standards (S1-S7) or 100ul (diluted or undiluted) samples to appropriate wells. For the Blank Control test, we recommend add 100ul Diluent Buffer to the well.
3) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at 37°C.
4) 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.

5) Add 100ul of above diluted detection antibody to each wells, cover the plate with a sealer, and then incubate for 90 mins at 37℃. Repeat the wash process as above Step 4.

6) Add 100ul TMB substrate to each well, put it on the oscillator to mix for 30s. Cover the plate with a new sealer, incubate at 37℃ for 10 to 20 mins. (Avoid sunlight).

   Note: The coloring time varies in different experimental conditions (temperature, humidity, ect.).

7) 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.

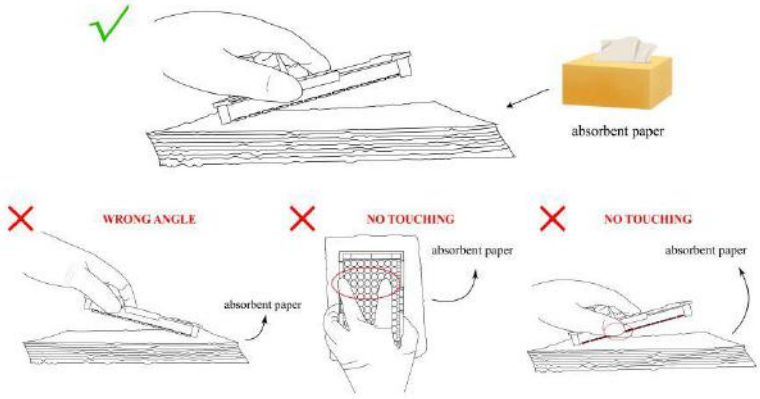
e) TMB is easily contaminated. please protect it from light and metal.

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 $R^2 \geqslant 0.95$.

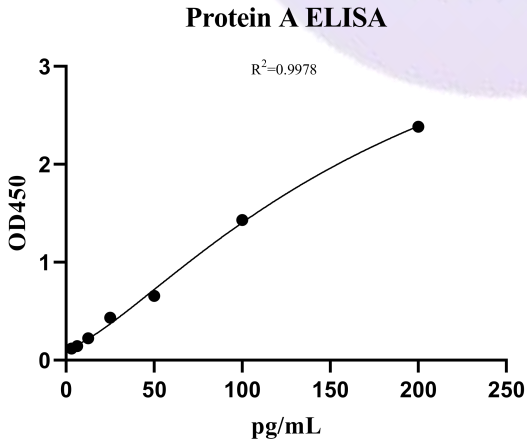
i) Schematic diagram of tapping the plate:




## 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




| pg/ml | OD450 |
|---|---|
| 200 | 2.385 |
| 100 | 1.43 |
| 50 | 0.655 |
| 25 | 0.433 |
| 12.5 | 0.223 |
| 6.25 | 0.143 |
| 3.125 | 0.118 |

## 13. QUALITY CONTROL

1) Intra Variation%: 4-8
2) Inter Variation%: 8-10
3) Recovery%: 67.36-115.92
4) Linearity:

| Dilution ratio | Range % | Average Linearity % |
|---|---|---|
| 4 | 98.25-105.77 | 102.01 |
| 40 | 100.68-109.74 | 105.21 |

5) Sensitivity: 1.4pg/ml
6) Specificity/Cross-reactivity:

| Sample | Cross reactivity（%） |
|---|---|
| Bovine IgG | --- |
| Goat IgG | --- |
| Human IgG | --- |

7) 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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