京天成生物技术（北京）有限公司
AbMax Biotechnology Co., LTD
99Kechuang 14th Street, Building 18,
2-201B, BDA, Beijing, China. 101111
Tel: 86-10-59755729, Fax: 86-10-59755718
E-mail: info@antibodychina.com
Website: www.antibodychina.com

Protein A Quantitation Kit (ELISA)

【Generic Name】

Protein A Quantitation Kit (ELISA)
Cat#: 17-0084

【Intended Use】

To detect the contents of Protein A in the samples.

【Principle】

This product adopts the principle of Double Antibody Sandwich Method (Sandwich Elisa). The flat-bottom 96-well plates are coated with anti-Protein A antibody. After adding the samples, the anti- Protein A antibody labeled with HRP is used for detection. The content of the PT in the blood samples can be detected by the degree of TMB color development.

【Materials and Reagents】

1. Coated plate, 12 wells × 8 strips
2. Enzyme conjugate, 120 μL×1 tube (diluted 100 times for use)
3. Sample solution, 30ml (contains hIgG, BSA, preservative) ×1 vial
4. Protein A standards ×5 tubes (0 ng/ml, 0.25 ng/ml, 1 ng/ml, 4 ng/ml, 16 ng/ml)
5. Enzyme solution, 30ml (contains BSA, preservative) ×1 vial
6. 20× Washing Buffer, 50mL×1 vial
7. Substrate Solution A, 7mL×1 vial
8. Substrate Solution B, 7mL×1 vial
9. Termination Solution, 7mL×1 vial
10. Sealing plate film, 2 pieces
11. Instruction book

【Storage】

2-8℃; Avoid light. Valid for six months.

【Protocol for Detection】

1. Equilibration
   Equilibrate the required reagents at room temperature (18~25℃) for 30 minutes.
2. Dosing solution: Please configure the reagents before use.

2.1 1×washing buffer: Take 1 vial of 20×washing buffer, dilute it to 1000ml with deionized water, mix well for later use.

2.2 Sample dilution: Dilute the sample by sample solution. For antibody drugs, 1:100 is suggested as the initial ratio.

2.3 Enzyme solution: Dilute the enzyme conjugate 100 times by the solution buffer, mix well.

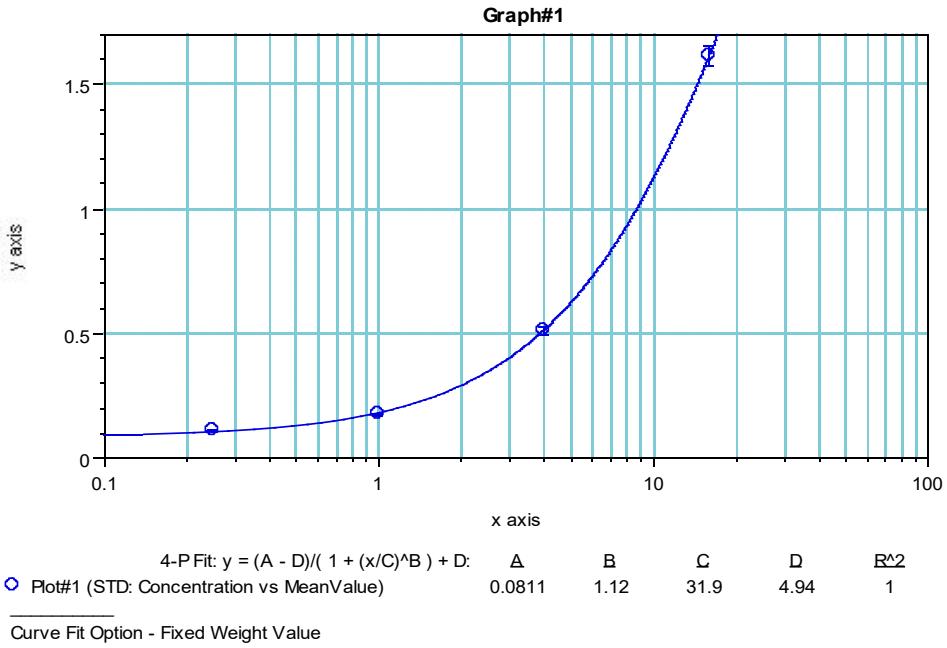
3. Adding standard and samples
   Remove the coated plate from the sealed bag and dilute the standard to the different concentrations. After adding 100μl of standard or sample to each well (including 0 ng/ml), seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 90 minutes
4. Washing
   Discard the liquid in each well, fill the microwells (350μl/well) with 1× washing buffer, and discard the liquid in the wells after 30 seconds. Repeat these steps for 3 times, then pat the plate on the paper towel after the last wash.
5. Adding enzyme solution
   Add the enzyme solution to the microplate (100μl per well). Seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes.
6. Washing
   Discard the liquid in each well, fill the microwells (350μl/well) with 1× washing buffer, and discard the liquid in the wells after 30 seconds. Repeat these steps for 5 times, then pat the plate on the paper towel after the last wash.
7. Coloring
   Add 50μl each of Substrate Coloring Solution A and B into each well. Mix well with gentle tapping. Then incubate the plate at room temperature for 5-10 minutes in the dark.
8. Termination
   Terminate the reaction by adding 50μl of 0.2M H_2SO_4 into each well and mix gently. Set the main wavelength of the microplate reader at 450nm and the reference wavelength at 630nm. Measure the absorbance (OD value) of each well.

【Data Analysis】

It is recommended to adopt the fitting method of four parameters or double logarithm for fitting and calculation.

Standard Curve Concentration	OD Value			Mean value
16	1.573	1.653	1.612	1.613
4	0.495	0.527	0.511	0.511
1	0.171	0.174	0.179	0.175
0.25	0.104	0.112	0.112	0.109
0	0.078	0.086	0.066	0.077

After four-parameter fitting of the concentrations and OD values, the curve equation is as follows:



【Product Performance Index】

1. Linear range: 0.25～16ng/ml
2. Detection limit: ≤0.1ng/ml
3. Accuracy：CV% ≤15% (n=10)

【Limitations】

1. This kit is only used to detect the content of Protein A antigen in the samples.
2. Results out of the measurement range of the kit are unreliable.
3. Severe hemolysis, chyle, and bilirubin samples may cause abnormal test results.
4. This kit is developed for in vitro research only

【Caution】

1. Avoid cross contamination.
2. Follow reader measure as a standard.
3. All samples and buffers should be added or removed with pipette.
4. Do not mix reagents from different batches.