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Pertussis Vaccine (PRN) Quantitation Kit (ELISA)

【Generic Name】

Pertussis (PRN) Quantitation ELISA Kit
Cat#: 17-0037

【Intended Use】

To detect the contents of PRN 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-PRN antibody. After adding the samples, the anti-PRN monoclonal antibody labeled with HRP is used for detection. The content of the PRN 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. BSA, 3G ×1 pack
4. 20× Washing Buffer, 50mL×1 vial
5. Substrate Solution A, 7mL×1 vial
6. Substrate Solution B, 7mL×1 vial
7. Termination Solution, 7mL×1 vial
8. Sealing plate film, 2 pieces
9. Instruction book

【Storage】

Enzyme conjugate: stored at -20℃;
Other components: 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 Dilution buffer (3% BSA-PBS): Dissolve BSA (3g/pack) completely into 100ml of the prepared 1×washing buffer (step 2.1), mix well for later use.

2.3 Enzyme solution: Take the required enzyme conjugate, dilute it 100 times with the solution buffer prepared in step 2.2, mix well for later use.

2.4 Sample solution (1% BSA-PBS): Dilute the 3% BSA prepared in step 2 to 1% BSA with the 1x washing buffer prepared in step 1, 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 negative control), seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 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
   After diluting the enzyme conjugate 100 times with the enzyme solution buffer, add the 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
   Repeat step 4.
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 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.

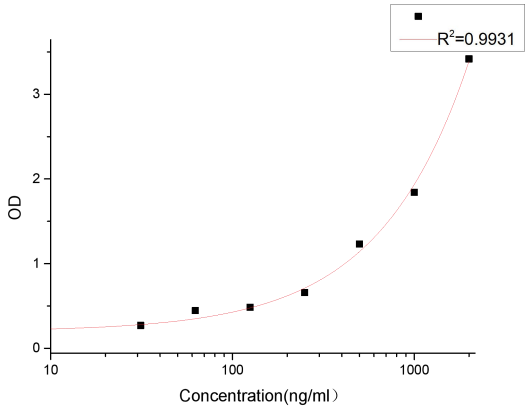
【Data Analysis】

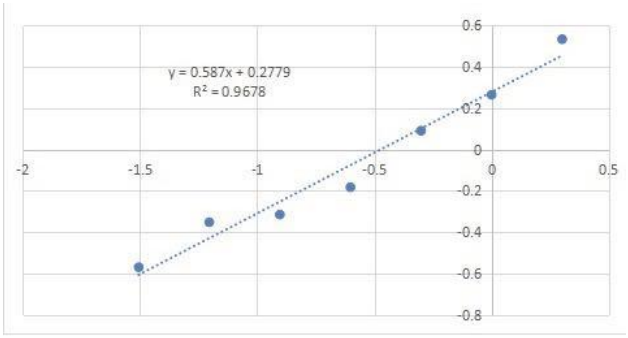
It is recommended to adopt the fitting method of four parameters or double logarithm for fitting and calculation.

Standard Curve Concentration	OD Value		Mean Value
2000	3. 620	3. 215	3. 418
1000	1. 878	1. 803	1. 841
500	1. 346	1. 119	1. 233
250	0. 688	0. 627	0. 658
125	0. 515	0. 454	0. 485

62. 5	0. 472	0. 418	0. 445
31. 25	0. 346	0. 193	0. 270
0	0. 113	0. 158	0. 136

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





【Product Performance Index】

1. Linear range: 31.25～2000ng/ml
2. Detection limit: ≤31.25ng/ml
3. Accuracy：CV% ≤15% (n=10)

【Limitations】

1. This kit is only used to detect the content of inactivated PRN antigen in blood 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.