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Pertussis Vaccine (ACT) Quantitation Kit (ELISA)

【Generic Name】

Pertussis Vaccine (ACT) Quantitation Kit (ELISA)
Cat#: 17-0100

【Intended Use】

To detect the contents of ACT 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-ACT polyclonal antibody. After adding the samples, the anti-ACT monoclonal antibody labeled with HRP is used for detection. The content of the ACT in the samples can be detected by the degree of TMB color development.

【Materials and Reagents】

1. Coated plate, 12 wells × 8 strips
2. Biotin conjugate, 120μl x 1 tube (diluted 100 times for use)
3. Enzyme conjugate, 120 μ L×1 tube (diluted 100 times for use)
4. Enzyme solution, 12m L×1 vial
5. BSA, 3G ×1 pack
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】

1. All components remain stable for six months under the temperature condition 2-8℃;
2. Return each component to 4°C immediately after use.

【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 bottle of 20×washing buffer, dilute it to 1000ml with deionized water, mix well.

2.2 3% BSA buffer: Dissolve BSA (3g/pack) completely into 100ml of the prepared 1×washing buffer (step 2.1), mix well.

2.3 Dilution buffer (1% BSA):

2.4 Biotin conjugate solution: Take the required Biotin conjugate, dilute it 100 times with the solution buffer prepared in step 2.2, mix well.

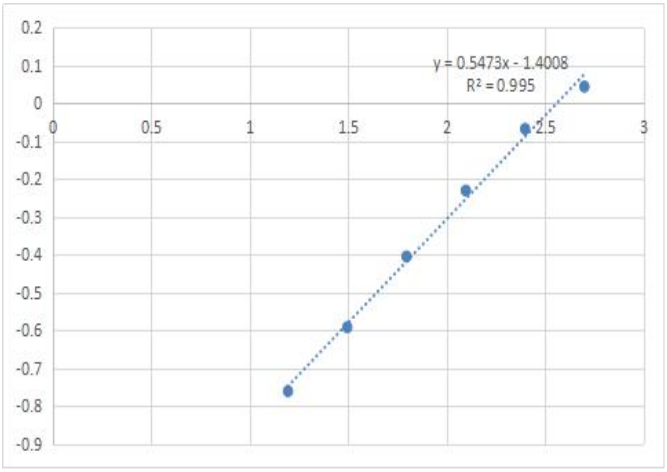
2.5 Enzyme solution: Take the required enzyme conjugate, dilute it 100 times with the solution buffer prepared in step 2.3, mix well.

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 the biotin conjugate solution
   Add the biotin conjugate 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. 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 45 minutes.
8. 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.
9. 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.
10. 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. After four-parameter fitting of the concentrations and OD values, the curve equation is as follows:

Standard Curve Concentration	OD Value		Mean Value
1	1.349	1.287	1.318
0.5	1.131	1.08	1.1055
0.25	0.852	0.858	0.855
0.125	0.59	0.585	0.5875
0.0625	0.403	0.384	0.3935
0.03125	0.266	0.246	0.256
0.015625	0.17	0.177	0.1735
NC	0.091	0.085	0.088



【Product Performance Index】

1. Linear range: 0.0156～0.5μg /ml
2. Detection limit: ≤0.03125μg/ml
3. Accuracy：CV% ≤15% (n=10)

【Limitations】

1. This kit is only used to detect the content of ACT antigen in 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.