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User's Manual

Bordetella pertussis (PT) Quantitation Kit

REF **DEIASL122**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Bordetella pertussis (PT) Quantitation Kit is designed for quantitatively detection of the contents of PT in the samples.

General Description

Whooping cough is a disease of the respiratory tracts which is caused by Bordetella pertussis bacteria. It is transmitted by airborne infection. The gramnegative Coccobacillus produces a series of biologically active molecules. The different compounds appear either during the pathogenesis or during the process of immunization against pertussis and show different effects. A characterisation has been made for the pertussis toxin (pt), the filamentery haemagglutinine (fha) and different lipopolysaccharides (lps). Pertussis shows a high rate of transmission (rates of infection of over 90 % have been found for non-vaccinated household members) and can cause severe diseases, especially for very young children. From 10749 patients under one year between 1980 and 1989 69 % were brought into hospital, 22 % suffered from pneumonia, 0.9 % showed an Encephalopathy and 0.6 % died. For older children and adults (including already vaccinated persons) the infection may be observed by an unspecified bronchitis or inflammation of the upper respiratory tracts. Even asymptomatic cases are quite common.

Principles of Testing

This product adopts the principle of Double Antibody Sandwich Method (Sandwich Elisa). The flat-bottom 96-well plates are coated with anti-PT rabbit polyclonal antibody. After adding the samples, the anti-PT monoclonal 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.

Reagents And Materials Provided

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

1. All components remain stable under the condition of 2-8°C;

2. Avoid light. Valid for six months

Reagent Preparation

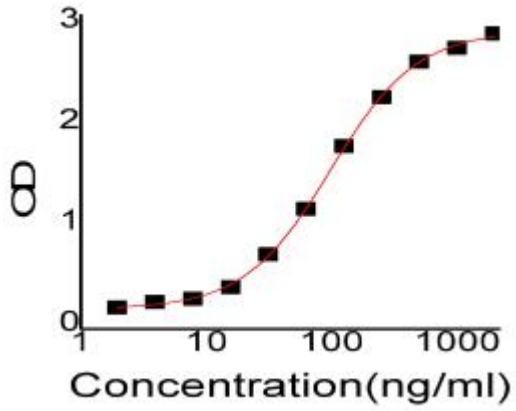
1. 1×washing buffer: Take 1 vial of 20×washing buffer, dilute it to 1000ml with deionized water, mix well for later use.
2. Dilution buffer (3% BSA-PBS): Dissolve BSA (3g/pack) completely into 100ml of the prepared 1×washing buffer (step 1), mix well for later use.
3. Enzyme solution: Take the required enzyme conjugate, dilute it 100 times with the solution buffer prepared in step 2, mix well for later use.
4. Sample solution (3% BSA-PBS): use the buffer prepared in step 2.

Assay Procedure

1. Equilibration: Equilibrate the required reagents at room temperature (18~25°C) for 30 minutes.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.

Typical Standard Curve

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



Precision

CV% ≤15% (n=10)

Detection Range

31.25~2000ng/ml

Detection Limit

Detection limit: ≤31.25ng/ml

Precautions

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.

Limitations

1. This kit is only used to detect the content of inactivated PT 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.