PERTUSSIS TOXIN
200-500 µg/mL

SSI DIAGNOSTICA

for *in vitro* diagnostic use

Pertussis toxin (PT) is for the quantitative measurement of antibodies against *Bordetella pertussis* in human serum samples.

Application

B. pertussis is only present in the airways for the first few weeks after the infection, but the symptoms last for a longer period due to presence of the toxin and several other virulence factors. Diagnostic methods detecting the bacterium itself (culture and PCR) are therefore only useful early in the infection. Serology is however very efficient for diagnosis of patients with prolonged cough. PT is the recommended antigen for pertussis ELISA as there is no risk of cross-reactions with other bacterial antigens.

Description

PT is available in volume of 100 µL in concentration ranging between 200 µg/mL and 500 µg/mL. PT is produced at AJ Vaccines A/S for use in child vaccinations and therefore it is highly purified. The PT for use in ELISA is in its active form though it is inactivated before use in the vaccination.

Principle

The PT is used as a coating agent in a traditional ELISA setup. It is recommended to use the below procedure.

Limitations

PT is intended to be used according to the published ELISA procedure in order to prevent false positives due to heat-treated human sera.
Diagnosis of recently pertussis vaccinated individuals (< 2 years prior) is not possible.
Use of the ELISA for evaluation of vaccination status and/or protective level is not possible.
Detection of antibody response to *Bordetella parapertussis* is not possible.

Materials Required but not Provided

- Maxisorp ELISA plate (NUNC®)
- Sealing tape (NUNC®)
- Human standard antiserum: NIBSC International standard PT-IgG 335 IU/mL.
- Coating buffer (1.13 g Na_2CO_3 + 3.30 g $NaHCO_3$ + 2 mL 10 % NaN_3 + 1 L sterile MilliQ water ; adjust to pH 9,6); store for 6 months at 2-8 °C.
- Washing buffer (1 L PBS pH 7,4 + 1 mL Tween 20 ; adjust to pH 7,4); store at room temperature for 1 week.
- Blocking buffer (1 g skim milk powder + 100 mL washing buffer); use the same day.
- Dilution buffer (90 mL washing buffer + 10 mL blocking buffer); use the same day.
- Rabbit-Anti-Human IgG HRP (DAKO® P0214)
- TMB One substrate (Kem-En-Tec Diagnostics)
- 1M Sulphuric acid

Procedure

Controls and unknown should be assayed in duplicates. Heat-treated sera can be analyzed with this method without the risk of false-positive results.

1. Dilute PT in cold coating buffer to the final concentration of 0,6 µg/mL (example for 12 mL dilution: $\frac{0.6\ \mu g/mL \times 12\ mL}{conc(PT)\ \mu g/mL}$ = X mL of PT in (12-X) mL coating buffer).
2. Add 100 µL diluted PT to each well of a Maxisorp ELISA plate (NUNC®). Apply the sealing tape and place the ELISA plate in a plastic bag.
3. Incubate 1 hour at 37 °C or at room temperature overnight.
4. Aspirate and wash 3 times (40 sec. soak/wash) with 250 µL washing buffer/well.
5. Add 200 µL blocking buffer to each well and incubate 30 min at room temperature.
6. Make a 2-fold dilution of the human standard antiserum in dilution buffer (1:1,000, 1:2,000, 1:4,000, 1:8,000, 1:16,000).
7. Aspirate and wash 3 times (40 sec. soak/wash) with 250 µL washing buffer/well.
8. Add 100 µL diluted human standard antiserum (1:2000, 1:4000, 1:8000, 1:16000) and patient sample (1:1000) to each defined well. Add 100 µL dilution buffer to the blank control well.
9. Incubate 1 hour at room temperature.
10. Aspirate and wash 3 times (40 sec. soak/wash) with 250 µL washing buffer/well.
11. Dilute 10 µL Rabbit-Anti-Human IgG HRP in 25 mL washing buffer. Add 100 µL of the dilution to each well.
12. Incubate 30 min at room temperature.
13. Aspirate and wash 3 times (40 sec. soak/wash) with 250 µL washing buffer/well.
14. Add 100 µL TMB One substrate. Incubate for exactly 15 min. at room temperature (no sealing).
15. Add 100 µL 1M sulphuric acid to each well (ends the reaction).
16. Read the absorbance within 10 min. using an ELISA reader set at 450 nm and 620 nm. The result is obtained by subtracting OD_{620} from OD_{450}.

The dilution series of the human standard antisera is used to make a log-log linear standard curve. All samples are calculated by the regression equation from the standard curve.
Results are valid if the CV % (coefficient of variance) for duplicate samples is lower than 20 %. Higher CV % values are allowed if the difference between the duplicate samples is less than 0.05 in OD. Results with OD readings above 2.0 and below 0.1 are outside the log-log linear range and should be interpreted as "higher than" or "lower than" the corresponding IU/mL values from the standard curve of these two OD values.
Cut-off values should be determined for each country or region as the general

antibody levels can vary between populations. Cut-offs in the range of 75 - 125 IU/mL is frequently used worldwide.

Storage and Shelf Life

Store at 2-8 °C (in a dark place). Expiry date is printed on the package. Do not freeze (if PT has accidentally been frozen, it should not be used).

References

1. Dalby T, Seier-Petersen M, Kristiansen MP et al. 2009. Problem solved: a modified enzyme-linked immunosorbent assay for detection of human antibodies to pertussis toxin eliminates false-positive results occurring at analysis of heat-treated sera. Diagnostic Microbiol Infect Dis 63: 354–360.
2. Guiso, N., G. Berbers, N. K. Fry, Q. He, M. Riffelmann, and C. H. Wirsing von König. 2010. What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. Eur. J Clin Microbiol Infect Dis.

Information and ordering

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