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User's Manual

Bordetella pertussis IgG ELISA Kit

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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

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PRODUCT INFORMATION

Intended Use

The Bordetella pertussis IgG Enzyme Immunoassay Kit provides materials for the qualitative and semiquantitative determination of IgG-class antibodies to Bordetella pertussis and Bordetella pertussis toxin in serum and plasma (EDTA-, heparin- or citrate plasma).

General Description

Bordetella species are non-spore-forming encapsulated bipolar, coccoid (pale-staining) Gram-negative bacilli (about 0.3 - 0.5 µm thick and 1 µm long). The genus consists of the human parasites B. pertussis and B. parapertussis, and B. bronchiseptica which cause enzootic infections in various wild and domestic animal species.

Bordetella pertussis produces a single-disease syndrome in man known as pertussis or whooping cough. It is a highly contagious childhood disease (approx. 80% of cases occur before the age of 5 years) which is transmitted by respiratory contact and is associated with a high mortality rate (about 1-2% in the first year of life, later on about 1%).

In the absence of immunization, essentially no one escapes pertussis. Clinical pertussis is followed by natural acquired immunity which is long-lasting but not permanent. The distribution of the disease is worldwide, though clearly modified by immunization and other poorly defined social, economic, and nutritional factors. In most countries an active vaccination is recommended. Usually the immunization preparation is combined with diphtheria and tetanus toxoids.

Principles of Testing

The Bordetella pertussis IgG ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Microtiter wells as a solid phase are coated with Bordetella pertussis and Bordetella pertussis toxin antigen. Diluted patient specimens and ready-for-use controls are pipetted into these wells. During incubation Bordetella pertussis and Bordetella pertussis toxin-specific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of Bordetella pertussis and Bordetella pertussis toxinspecific IgG antibody in the patient specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Reagents And Materials Provided

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells;

Wells coated with Bordetella pertussis and Bordetella pertussis toxin antigen. (incl. 1 strip holder and 1 cover

foil)

2. Sample Diluent *, 1 vial, 100 mL, ready to use,
colored yellow; pH 7.2 ± 0.2.

3. Pos. Control *, 1 vial, 2.0 mL, ready to use;
colored yellow, red cap.

4. Neg. Control *, 1 vial, 2.0 mL, ready to use;
colored yellow, yellow cap.

5. Cut-off Control *, 1 vial, 2.0 mL, ready to use;
colored yellow, black cap.

6. Enzyme Conjugate *, 1 vial, 20 mL, ready to use,
colored red, antibody to human IgG conjugated to horseradish peroxidase.

7. Substrate Solution, 1 vial, 14 mL, ready to use,
Tetramethylbenzidine (TMB).

8. Stop Solution, 1 vial, 14 mL, ready to use,
contains 0.2 mol/L H_2SO_4,
Avoid contact with the stop solution. It may cause skin irritations and burns.

9. Wash Solution *, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5± 0.1
see „Preparation of Reagents".

* contain non-mercury preservative

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450/620 nm ±10 nm) (e.g. the DRG Instruments Microtiter Plate Reader)
2. Calibrated variable precision micropipettes
3. Incubator 37 °C
4. Manual or automatic equipment for rinsing wells
5. Vortex tube mixer
6. Deionised or (freshly) distilled water
7. Timer
8. Absorbent paper

Storage

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

Specimen Collection And Preparation

Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma: Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Specimen Storage

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying. Specimens held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

Prior to assaying dilute each patient specimen 1+100 with Sample Diluent; e.g. 10 µL of specimen + 1 mL of Sample Diluent mix well, let stand for 15 minutes mix well before use.

Please note: Controls are ready for use and must not be diluted!

Plate Preparation

1. It is very important to bring all reagents, samples and controls to room temperature before starting the test run!
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
7. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells. During incubation cover microtiter strips with foil to avoid evaporation.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute Wash Solution 1+19 (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted

wash solution has a pH value of 7.2 ± 0.2.

Consumption: ~ 5 mL per determination.

Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use.

The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

Assay Procedure

Prior to commencing the assay, dilute Wash Solution, prepare patient samples as described above, mix well before pipette and establish carefully the distribution and identification plan supplied in the kit for all specimens and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well (e.g. A1) for the substrate blank,

1 well (e.g. B1) for the Neg. Control,

2 wells (e.g. C1+D1) for the Cut-off Control and

1 well (e.g. E1) for the Pos. Control.

It is left to the user to determine controls and patient samples in duplicate.

2. Dispense

100 µL of Neg. Control into well B1

100 µL of Cut-off Control into wells C1 and D1

100 µL of Pos. Control into well E1 and

100 µL of each diluted , mixed sample with new disposable tips into appropriate wells.

Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for 60 minutes at 37 °C.
4. Briskly shake out the contents of the wells.

Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense 100 µL Enzyme Conjugate into each well, except A1.
6. Incubate for 30 minutes at room temperature (20 °C to 25 °C).

Do not expose to direct sun light!

7. Briskly shake out the contents of the wells.

Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

8. Add 100 µL of Substrate Solution into all wells.

9. Incubate for exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark.
10. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.

Any blue color developed during the incubation turns into yellow.

Note: Highly positive patient samples can cause dark precipitates of the chromogen!

11. Read the optical density at 450/620 nm with a microtiter plate reader within 30 minutes after adding the Stop Solution.

Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or Creative Diagnostics directly.

The test run may be considered valid provided the following criteria are met:

Substrate blank in A1: Absorbance value lower than 0.100

Neg. Control in B1: Absorbance value lower than 0.200

Cut-off Control in C1/D1 : Absorbance value between 0.350 – 0.850

Pos. Control in E1: Absorbance value between 0.650 – 3.000

Calculation

Adjust the ELISA microplate or microstrip reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

Interpretation Of Results

Mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in C1/D1).

Example: $(0.54 + 0.56)/2 = 0.55 = CO$

POSITIVE Patient (mean) absorbance values more than 10 % above CO

(Mean OD patient> 1.1 x CO)

GREY ZONE Patient (mean) absorbance values from 10 % above to 10 % below CO

repeat test 2 - 4 weeks later - with new patient samples

(0.9 x CO ≤ Mean OD patient ≤ 1.1 x CO)

Results in the second test again in the grey zone NEGATIVE

NEGATIVE Patient (mean) absorbance values more than 10 % below CO

(Mean OD patient < 0.9 x CO)

Patient (mean) absorbance value x 10 = [DRG Units = DU]

CO

Example: 1.580 x 10 = 29 DU

0.55

Interpretation of Results

Cut-off value: 10 DU

Grey zone: 9 - 11 DU

Negative: < 9 DU

Positive:> 11 DU

Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 95.49 %.

Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 95.24 %.

Interferences

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Precautions

1. Before starting the assay, read the instructions completely and carefully. Use the valid version of the

package insert provided with the kit. Be sure that everything is understood.

2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Avoid contact with Stop Solution containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
4. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
5. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
6. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
7. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
8. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
9. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
10. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
11. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
12. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
13. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
14. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
15. Do not use reagents beyond expiry date as shown on the kit labels.
16. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
17. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
19. For information on hazardous substances included in the kit please refer to Safety Data Sheets.

Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. In immunocompromised patients and newborns serological data only have restricted value.