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ab247192

Bordetella pertussis

toxin (PT) IgA ELISA kit

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For the quantitative determination of IgG class antibodies against Bordetella pertussis toxin in human serum or plasma (citrate, heparin).

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

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1. Overview

Bordetella pertussis toxin (PT) IgA ELISA kit (ab247192) is designed for the quantitative determination of IgA class antibodies against *Bordetella pertussis* toxin antigens in human serum or plasma (citrate, heparin).

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

Bordetella pertussis is a respiratory pathogen that causes pertussis, commonly known as whooping cough, a localized infection of the ciliated epithelium of the bronchial tree. Pertussis is characterized by a prolonged paroxysmal cough often accompanied by an inspiratory whoop.

The disease affects mainly children, but adults have also been increasingly reported to be affected. The pathogen produces toxins which cause local damage to the cilia of epithelial cells, which leads to prolonged illness and pertussis. Disease presentation varies with age and history of previous exposure or vaccination. Severe disease is infrequent in healthy, vaccinated persons. Infants, particularly those who have not received the primary vaccination series against pertussis, are at risk for complications and mortality.

2. Protocol Summary

Prepare all reagents, samples, standards and controls as instructed



Add 100 μ L control, standard or sample to appropriate wells



Incubate for 1 hour at 37°C



Aspirate and wash each well three times with 300 μ L 1X Washing Solution



Add 100 μ L of HRP conjugate to each well. Incubate for 30 minutes at room temperature. Repeat the washing steps.



Add 100 μ L TMB Substrate Solution to each well and incubate for 30 minutes at room temperature.



Add 100 μ L Stop Solution and read OD at 450/620 nm within 30 minutes after addition of the stop solution.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
20X Washing Solution	50 mL	+4°C
Cover Foil	1 unit	+4°C
Stop Solution	15 mL	+4°C
TMB Substrate Solution	15 mL	+4°C
B. pertussis toxin (PT) Coated Microplate (IgA)	1 unit	+4°C
anti-human IgA HRP conjugate	20 mL	+4°C
IgA high Control – concentration on the label	2 mL	+4°C
IgA low Control – concentration on the label	2 mL	+4°C
IgA Sample Diluent	100 mL	+4°C
B. pertussis toxin (PT) IgA Standard A – 0 IU/mL	2 mL	+4°C
B. pertussis toxin (PT) IgA Standard B – 10 IU/mL	2 mL	+4°C
B. pertussis toxin (PT) IgA Standard C – 25 IU/mL	2 mL	+4°C
B. pertussis toxin (PT) IgA Standard D – 50 IU/mL	2 mL	+4°C

The standards are calibrated in accordance with the "1st WHO International Standard Pertussis antiserum (human)", Code 06/140, of the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450/620 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Plate shaker for all incubation steps.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 20X Washing Solution:

Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL combine 10 mL 20X with 190 mL deionized water. Mix thoroughly and gently. The diluted buffer is stable for 5 days at room temperature. In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

B. pertussis toxin (PT) Coated Microplate (IgA):

The break-apart snap-off strips are coated with B. pertussis antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 4 °C.

10. Sample Preparation

- Use human serum or plasma (citrate, heparin) samples with this assay.
- If the assay is performed within 5 days after sample collection, the samples should be kept at 4°C; otherwise they should be aliquoted and stored deep-frozen (-70°C). If samples are stored frozen, mix thawed samples well before testing.
- Avoid repeated freezing and thawing.
- Heat inactivation of samples is not recommended.

11. Sample Dilution

- Before assaying, all samples should be diluted 1:100 with IgA Sample Diluent. Dispense 10 µL sample and 990 µL IgA Sample Diluent into tubes to obtain a 1:100 dilution and thoroughly mix with a Vortex.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - We recommend that you assay all controls and samples in duplicate.
- 12.1 Prepare all reagents, controls and samples as directed in the previous sections.
 - 12.2 Use the plate layout to plan the location for all controls and samples.
 - 12.3 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
 - 12.4 Add 100 µL of all diluted samples and controls to appropriate wells. Leave well A1 as the Substrate blank. Cover the wells with the cover foil.
 - 12.5 Incubate for 1 hour at 37°C.
 - 12.6 When incubation is completed, remove the foil, aspirate the content of the wells and wash each well 3 x 300 µL with 1X Washing Solution. Avoid overflows from the reaction wells. The interval between washing and aspiration should be less than 5 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
 - 12.7 Add 100 µL of HRP conjugate to each well, except A1, and incubate for 30 minutes in the dark at room temperature.
 - 12.8 Repeat the washing steps as per 12.6.
 - 12.9 Add 100 µL of TMB Substrate Solution to each well. Incubate for exactly 30 minutes at room temperature in the dark. A blue color occurs due to an enzymatic reaction.
 - 12.10 Add 100 µL of Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
 - 12.11 Measure the absorbance at 450/620 nm within 30 minutes after addition of the Stop Solution.

13.Measurement

13.1 Adjust the microplate reader to zero using the Substrate blank.

Δ Note: If due to technical reasons that the microplate reader cannot be adjusted to zero using the blank, then subtract its absorbance value from all other absorbance values measured to obtain reliable results.

13.2 Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and sample in the plate layout.

13.3 Biochromatic measurement using a reference wavelength of 620 nm is recommended.

13.4 Where applicable calculate the mean absorbance values for all duplicates.

13.5 Run validation criteria:

- For an assay to be considered valid, the following criteria must be met.
- If these criteria are not met, the test is not valid and must be repeated.

Controls	Absorbance value
Substrate Blank	< 0.1
Standard A	< 0.2
Standard B	> Standard A
Standard C	> Standard B
Standard D	> 1.0
Low Control	Result in IU/MI within range indicated on the label
High Control	Result in IU/MI within range indicated on the label

Standard A < Standard B < Standard C < Standard D

14. Calculations

- 14.1 To obtain quantitative results in IU/mL plot the mean absorbance values of the 4 Standards A - D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 10, 25 and 50 IU/mL) and draw a standard curve (absorbance values on the y-axis, concentrations on the x-axis).
- 14.2 Read results from this calibration curve employing the mean absorbance values of each patient sample and control.
- 14.3 For the calculation of the standard-curve, the mathematical Point to Point function should be used.

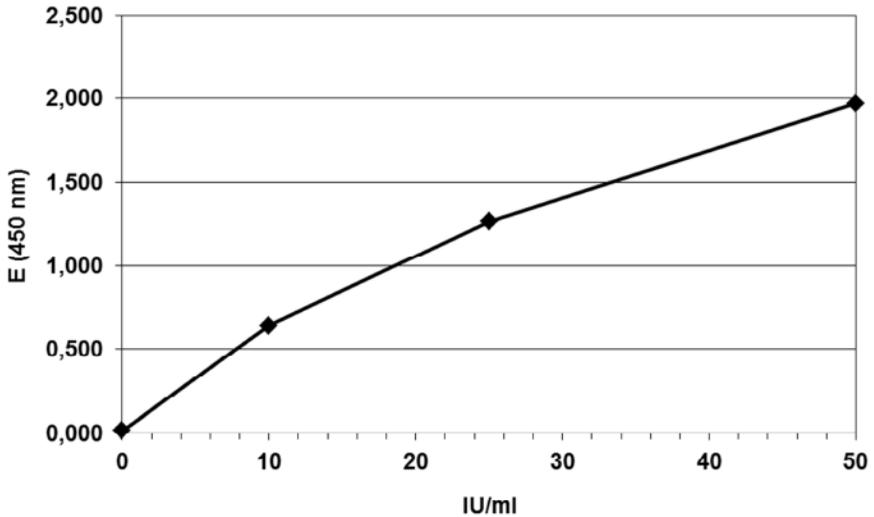


Figure 1. Typical Standard Curve.

15. Interpretation of Results

According to recent literature and recommendations from reference laboratories the following interpretation of results is recommended:

Concentration	Interpretation of results
< 12 IU/mL	Not indicative of recent contact
≥ 12 IU/mL	Indicative of recent contact

Diagnosis of an infectious disease should not be established based on a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.

Serology	Significance
IgG	Characteristic of the secondary antibody response. May persist for several years. High IgG titer with low IgM titer: → may indicate a past infection.
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early during the infection.

16. Typical Sample Values

PRECISION –

- The reproducibility of the kit was determined by comparing a minimum of 23 replicates of 3 different samples in one assay (intra-assay) and by comparing 3 different samples assayed in 12 different runs (inter-assay).
- Acceptance Criterion: CV < 15 %

	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
n =	23	24	24	12	12	12
Mean (OD)	0.547	1.954	1.048	5.19	16.29	6.23
CV (%)	8.01	2.03	2.66	7.28	4.37	8.17

DIAGNOSTIC SPECIFICITY –

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

It is 90.91% (95% confidence interval: 75.67% - 98.08%).

DIAGNOSTIC SENSITIVITY –

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

It is 100.0% (95% confidence interval: 69.15% - 100.0%).

INTERFERENCES –

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

CROSS REACTIVITY –

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters (including antibodies to several respiratory pathogens) did not reveal evidence of false-positive results due to cross-reactions. The pathogens tested were

Adenovirus, Chlamydia trachomatis, Chlamydophila pneumoniae, EBV, Helicobacter pylori, Influenzavirus A, Legionella pneumophila, Mycoplasma pneumoniae, Toxoplasma gondii, VZV.

MEASUREMENT RANGE –

The measurement range is 1.56 IU/mL - 50 IU/mL.

It is defined by the analytical sensitivity (Limit of Detection: 1.56 IU/mL) and the concentration of the highest standard (50 IU/mL).

LIMITATIONS OF THE PROCEDURE –

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

Analytical Sensitivity (Limit of Detection) –

The terms Limit of Blank (LoB) and Limit of Detection (LoD) are used to describe the smallest concentration that can be reliably measured by an analytical procedure. The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoD is the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible.

The LoD or analytical sensitivity was determined according to the approved guideline CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation".

For this the zero standard, Standard A (= blank) and one low concentration sample were determined 60-fold on the Bordetella pertussis toxin (PT) IgA ELISA.

The LoD was calculated according to the following formula:

$$\begin{aligned} \text{LoD} &= \text{LoB} + 1.64 \times \text{SD}_{\text{low concentration sample}} = \\ &= \text{mean}_{\text{blank}} + 1.645 \times \text{SD}_{\text{blank}} + 1.645 \times \text{SD}_{\text{low concentration sample}} \end{aligned}$$

Specification: Acceptance criterion = LoD ≤ 5 IU/ML

Results:

	Absorbance (450 nm)	
	Standard A	Low concentration sample
Mean	0.01038	0.06033
SD	0.00255	0.01009
	LoB = 0.01457	LoD = 0.09342 = 1.56 IU/mL

The Limit of Detection (LoD) was estimated at 1.56 IU/ml. Therefore, the acceptance criterion is met.

17. Notes

Technical Support

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Austria

wissenschaftlicherdienst@abcam.com | 019-288-259

France

supportscientifique@abcam.com | 01.46.94.62.96

Germany

wissenschaftlicherdienst@abcam.com | 030-896-779-154

Spain

soportecientifico@abcam.com | 91-114-65-60

Switzerland

technical@abcam.com

Deutsch: 043-501-64-24 | Français: 061-500-05-30

UK, EU and ROW

technical@abcam.com | +44(0)1223-696000

Canada

ca.technical@abcam.com | 877-749-8807

US and Latin America

us.technical@abcam.com | 888-772-2226

Asia Pacific

hk.technical@abcam.com | (852) 2603-6823

China

cn.technical@abcam.com | +86-21-2070-0500 | 400-921-0189

Japan

technical@abcam.co.jp | +81-(0)3-6231-0940

Singapore

sg.technical@abcam.com | 800 188-5244

Australia

au.technical@abcam.com | +61-(0)3-8652-1450

New Zealand

nz.technical@abcam.com | +64-(0)9-909-7829