ab108709

Bordetella pertussis IgG
Human ELISA Kit

Instructions for Use

For the qualitative measurement IgG class antibodies against Bordetella pertussis and Bordetella pertussis toxin in Human serum or plasma (citrate).

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

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1. Introduction

ab108709 Bordetella pertussis IgG Human ELISA Kit is intended for the qualitative determination of IgG class antibodies against Bordetella pertussis and Bordetella pertussis toxin in Human serum or plasma (citrate).

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 and B.Avium which cause enzootic infections in various wild and domestic animal species. B. pertussis is the classical exciter of pertussis and exists only in ill people; B. parapertussis causes 5-20% of a milder and often clinical unapparent form of pertussis. B.Bronchiseptica has seldomly (e.g. close contact with animals) Human pathogenic significance as opportunistic secondary exciter in mixed infections (bronchitis, pneumonia, wound infection).
2. Assay Summary

*ab108709 is for the qualitative immunoenzymatic determination of IgG-class antibodies against Bordetella pertussis and is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.*

Microtiter strip wells are precoated with Bordetella pertussis/toxin antigens to bind corresponding antibodies of the specimen.

After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labeled anti-Human IgG conjugate is added. This conjugate binds to the captured Bordetella pertussis/toxin specific antibodies.

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 Bordetella pertussis/toxin specific IgG antibodies in the specimen.

Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorption at 450 nm is read using an ELISA microwell plate reader.
3. Kit Contents

- **Bordetella pertussis Coated Wells (IgG):** 12 break apart 8-well snap-off strips coated with Bordetella pertussis antigen; in resealable aluminum foil.
- **IgG Sample Diluent ***:** 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.)***: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Bordetella pertussis anti-IgG Conjugate***: 1 bottle containing 20 ml of peroxidase labeled Rabbit antibody to Human IgG; colored blue, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Bordetella pertussis IgG Positive Control***: 1 bottle containing 2 ml; colored yellow; ready to use; red cap.
- **Bordetella pertussis IgG Cut-off Control***: 1 bottle containing 3 ml; colored yellow; ready to use; green cap.
- **Bordetella pertussis IgG Negative Control***: 1 bottle containing 2 ml; colored yellow; ready to use; blue cap.
- Strip holder.
- 1 Cover foil.

* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4. Storage and Handling

The reagents are stable up to the expiry date stated on the label when stored at 2-8 °C.

5. Additional Materials Required

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 μl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer
6. Preparation of Reagents

1. It is very important to bring all reagents, samples and standards to room temperature (22-28°C) before starting the test run.

2. **Coated snap-off Strips:** The ready to use break apart snap-off strips are coated with Bordetella pertussis antigen. Store at 2-8°C. **Immediately after removal of strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 2-8 °C; stability until expiry date.**

3. **Bordetella pertussis anti-IgG Conjugate:** The bottle contains 20 ml of a solution with anti-Human IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. The solution is ready to use. Store at 2-8°C. **After first opening stability until expiry date when stored at 2-8 °C.**

4. **Controls:** The bottles labeled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and should be stored at 2-8°C. **After first opening stability until expiry date when stored at 2-8 °C.**

5. **IgG Sample Diluent:** The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the specimen. This ready to use solution should be stored at 2-8°C. **After first opening stability until expiry date when stored at 2-8 °C.**

6. **Washing Solution (20x conc.):** The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute
Washing Solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh and germ free redistilled water. The diluted buffer is stable for 5 days at room temperature. After first opening stability until expiry date when stored at 2-8 °C.

7. **TMB Substrate Solution:** The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and should be stored at 2-8°C away from the light. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability until expiry date when stored at 2-8 °C.

8. **Stop Solution:** The bottle contains 15 ml 0.2 M sulphuric acid solution. This ready to use solution should be stored at 2-8°C. After first opening stability until expiry date.

### 7. Preparation and Collection of Specimen

Use Human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -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.
**Sample Dilution:**

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10μl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

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**8. Assay Method**

**Test Preparation**

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300μl to 350μl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established. 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 negative control  
2 wells (e.g. C1+D1) for the cut-off control  
1 well (e.g. E1) for the positive control
• It is left to the user to determine controls and samples in duplicate, if necessary.
• Perform all assay steps in the order given and without any appreciable delays between the steps.
• A clean, disposable tip should be used for dispensing each standard and each sample.
• Adjust the incubator to 37° ± 1°C.

**Assay Procedure:**

1. Dispense 100 µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 seconds. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   
   *Note: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.*
5. Dispense 100µl Bordetella pertussis IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.** *Do not expose to direct sunlight.*

7. Repeat step 4.

8. Dispense 100µl TMB Substrate Solution into all wells.

9. **Incubate for exactly 15 min at room temperature in the dark.**

Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow. 

*Note: Highly positive samples can cause dark precipitates of the chromogen. These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.*

10. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

**Measurement:**

- Adjust the ELISA Microwell Plate Reader to zero using the substrate blank (e.g. 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 standard and 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.

9. Data Analysis

A. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

• **Substrate blank** in A1: Absorbance value < 0.100.
• **Negative control** in B1: Absorbance value < 0.200 and < cut-off.
• **Cut-off control** in C1 and D1: Absorbance value **0.150 – 1.300**.
• **Positive control** in E1: Absorbance value > cut-off.

If these criteria are not met, the test is not valid and must be repeated.
B. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

\[
\text{Example: Absorbance value Cut-off control } 0.39 + \text{ absorbance value Cut-off control } 0.37 = 0.76 / 2 = 0.38
\]

\[
\text{Cut-off } = 0.38
\]

C. Interpretation of Results

- Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.
- Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative. This is considered the grey zone. It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.
- Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.
D. Results in Abcam Units

Sample (mean) absorbance value x 10 = [Abcam Units = NTU]

Cut-off

Example: $1.216 \times 10 = 32$ NTU [Abcam Units = NTU]

Cut-off: 10 NTU
Grey zone: 9-11 NTU
Negative: <9 NTU
Positive: >11 NTU

E. Precision

<table>
<thead>
<tr>
<th>Interassay</th>
<th>n</th>
<th>Mean (NTU)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Serum</td>
<td>12</td>
<td>15</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>6.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intra assay</th>
<th>n</th>
<th>Mean (OD)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Serum</td>
<td>20</td>
<td>0.55</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.48</td>
<td>5.2</td>
</tr>
</tbody>
</table>
F. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 94%.

G. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 97.4%.

H. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

10. Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
## 11. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “contact us” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).
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