Instructions for Use

For the quantitative measurement of IgG class antibodies against Clostridium tetani toxin in Human serum or plasma (citrate).

This product is for research use only and is not intended for \textit{in vitro} diagnostic use.

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1. Introduction

ab108771 Clostridium tetani toxin IgG Human ELISA Kit is intended for the quantitative determination of IgG class antibodies against Clostridium tetani toxin in human serum or plasma (citrate).

Clostridia are spore-forming gram-positive bacteria. The round spores develop at the terminal end which results in a “tennis racket” like shape under the microscope. Tetanus develops only when spores of Clostridium tetani germinate under strict anaerobic conditions after gaining access to wounds and small lacerations. The clinical manifestation of the disease is not caused by the invasion of the exciter but by the secretion of a powerful neurotoxin (tetanospasmin). This toxin blocks the inhibition of the signal transduction and has a high affinity to the central nervous system. The consequence is hyper excitability of the muscles to external stimuli in combination with a principal increase of the muscle tonus without influence of consciousness.

Clostridium tetani is ubiquitous present in soil and intestine of humans and animals. Ingestion of bacteria or growth in the intestine of man or animal is without harm. The spores are extremely resistant towards heat and can stay infectious for a long period. The bacteria can get under the skin by even the smallest of wounds.
2. Assay Summary

*ab108771* is for the quantitative immunoenzymatic determination of IgG-class antibodies against Tetanus toxoid and is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with inactivated Tetanus toxoid antigens to bind corresponding antibodies of the specimen. Incubated for 1 hour at 37°C.

After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labeled anti-human IgG conjugate is added. Incubated at room temperature for 30 minutes. This conjugate binds to the captured Tetanus toxoid specific antibodies.

The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. Incubated at room temperature for 15 minutes. The intensity of this product is proportional to the amount of Tetanus toxoid 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

- **Clostridium tetani toxin Coated Wells (IgG):** 12 break apart 8-well snap-off strips coated with inactivated Tetanus toxoid antigen; in resealable aluminium 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 for washing the wells; pH 7.2 ± 0.2; white cap.

- **Clostridium tetani toxin anti-IgG Conjugate**: 1 bottle containing 20 ml of peroxidase labeled antibodies 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.

- **Clostridium tetani toxin IgG Standards***: 4 vials each containing 2 ml; ready to use.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Value (IU/ml)</th>
<th>Color</th>
<th>Cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0</td>
<td></td>
<td>blue</td>
</tr>
<tr>
<td>B</td>
<td>0.1</td>
<td></td>
<td>green</td>
</tr>
<tr>
<td>C</td>
<td>0.5</td>
<td></td>
<td>yellow</td>
</tr>
<tr>
<td>D</td>
<td>1.0</td>
<td></td>
<td>red</td>
</tr>
</tbody>
</table>

- **Strip holder:** 1
• **Cover foil**: 1

  * 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

It is very important to bring all reagents, samples and standards to room temperature (20-25°C) before starting the test run.

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

2. **Clostridium tetani toxin anti-IgG Conjugate**: The bottle contains 20 ml conjugate with the components 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 stable until expiry date when stored at 2-8 °C.*

3. **Standards**: The vials labeled with Standard A, B, C and D contain a ready to use standard solution. The concentration of the standards, calibrated in accordance with the “1st International Standard for TETANUS IMMUNOGLOBULIN, HUMAN” (Code TE-3) of the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK, are:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0 IU/ml</td>
</tr>
<tr>
<td>B</td>
<td>0.1 IU/ml</td>
</tr>
<tr>
<td>C</td>
<td>0.5 IU/ml</td>
</tr>
<tr>
<td>D</td>
<td>1.0 IU/ml</td>
</tr>
</tbody>
</table>
The solutions have to be stored at 2 - 8°C and contain 0.1% Kathon. **After first opening stable until expiry date when stored at 2 - 8°C.**

4. **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 has to be stored at 2 - 8°C. **After first opening stable until expiry date when stored at 2 - 8°C.**

5. **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. **Crystals in the solution disappear by warming up to 37 °C in a water bath. After first opening stability until expiry date when stored at 2-8°C.**

6. **TMB Substrate Solution:** The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to 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 stable until expiry date when stored at 2 - 8°C.**

7. **Stop Solution:** The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26), ready to use, store at 2 - 8°C. **After first opening stable 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.

For samples with expected antitoxin concentrations greater than Standard D (1.0 IU/ml) a second 1 + 1 dilution of this 1 + 100 diluted sample should be performed; e.g. 100 μl of first sample dilution + 100 μl of IgG sample diluent (mix well). Dilution factor: 2
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
4 wells (e.g. B1, C1 etc.) for Standard A, B, C and D.

It is recommended to determine samples in duplicate.

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 of each Standard (A, B, C and D) and diluted samples into the 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 >5sec. 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 Clostridium tetani toxin anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature (20 - 25°C).** *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 (20 - 25°C) in the dark.**
10. 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 as described under Sample Dilution is recommended.

11. 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 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 standard and sample.

*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. Assay Validation Criteria

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

Substrate blank in A1: Absorbance < 0.100
Standard A in B1: Absorbance < 0.200
Standard B in C1: Absorbance >0.150
Standard C in D1: Absorbance >0.500
Standard D in E1: Absorbance >1.000

Standard A < Standard B < Standard C < Standard D

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

B. Calculation of Results

In order to obtain quantitative results in IU/ml plot the (mean) absorbance values of the 4 Standards A, B, C and D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0.0, 0.1, 0.5 and 1.0 IU/ml) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).
Read results from this standard curve employing the (mean) absorbance values of each specimen and control.

**Note:** Readings of additionally (1+1) diluted samples must be multiplied by the appropriate dilution factor in order to obtain correct results! (Dilution: 1+1 = Dilution factor: 2).

All suitable computer programs available can be used for automated result reading and calculation.

**C. Typical Calibration Curve**
D. Interpretation of Results [IU/ml]

< 0.01  No antibody level

0.01 - 0.1  Low antibody level

0.11 – 0.5  Medium antibody level

> 1.0  High antibody level

E. Precision

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>n</th>
<th>Mean</th>
<th>Cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak pos. Serum</td>
<td>24</td>
<td>0.56</td>
<td>7.1</td>
</tr>
<tr>
<td>Pos.</td>
<td>24</td>
<td>1.24</td>
<td>4.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-assay</th>
<th>n</th>
<th>Mean (IU/ml)</th>
<th>Cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak pos. Serum</td>
<td>10</td>
<td>0.22</td>
<td>11.3</td>
</tr>
<tr>
<td>Pos.</td>
<td>10</td>
<td>0.54</td>
<td>7.7</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 > 95 %.

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G. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is > 95%.

H. Analytical Sensitivity

The analytical sensitivity – defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator – is <0.05 IU/ml.

I. 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>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</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>
</tbody>
</table>

Ensure proper sample reconstituted (if correctly reconstituted (if applicable).
<table>
<thead>
<tr>
<th>Problem Description</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<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>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<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>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<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>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) or phone (select “contact us” on www.abcam.com for the phone number for your region).
ab108771 Clostridium tetani toxin IgG Human ELISA Kit
Abcam in the USA
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA
Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

Abcam in Japan
Abcam KK
1-16-8 Nihonbashi
Kakigaracho,
Chuo-ku, Tokyo
103-0014
Japan
Tel: +81-(0)3-6231-094
Fax: +81-(0)3-6231-0941

Abcam in Europe
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK
Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

Abcam in Hong Kong
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong
Tel: (852) 2603-682
Fax: (852) 3016-1888