ab108727

Corynebacterium diphtheriae toxin 5S IgG Human ELISA Kit

Instructions for Use

For the quantitative measurement of IgG class antibodies against Corynebacterium diphtheriae toxin in Human serum or plasma (citrate)

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

*ab108727 Corynebacterium diphtheriae toxin 5S IgG Human ELISA Kit* is intended for the quantitative determination of IgG class antibodies against Corynebacterium diphtheriae toxin in Human serum or plasma (citrate).

Corynebacteria are aerobic non spore-forming gram-positive rods of irregular shape (0.5 –1 µm thick and 2-6 µm long). They comprise skin commensals, opportunist pathogens and several major pathogens, including Corynebacterium diphtheriae. In general, they are isolated from throat swabs on selective media containing tellurite. The bacterial infection caused by C. diphtheriae, Diphtheria, has two forms. Respiratory diphtheria is typically caused by toxin-producing (toxigenic) strains; cutaneous disease can be caused by either toxigenic or nontoxigenic strains. In the respiratory form of the disease, a membrane is formed; this membrane is usually visible on the throat or tonsils. Persons may die from asphyxiation when the membrane obstructs breathing. Other complications are caused by remote effects of the diphtheria toxin (myocarditis, nerve paralysis) Cutaneous diphtheria is usually mild, typically consisting of non-distinctive sores or shallow ulcers and only rarely involving toxic complications (1-2% of infections with toxigenic strains). Diphtheria was one of the most common causes of death among children during the prevaccine era.
2. Assay Summary

*ab108727 is for the quantitative immunoenzymatic determination of IgG-class antibodies against C. diphtheriae toxin and is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.*

Microtiter strip wells are precoated with inactivated specific Corynebacterium diphtheriae toxin (toxoid) antigens to bind corresponding antibodies of the specimen.

↓

After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-Human IgG conjugate is added. This conjugate binds to the captured C. diphtheriae 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 C. diphtheriae 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

- C. diphtheriae toxin Coated Wells (IgG): 12 break-apart 8-well snap-off strips coated with C. diphtheriae toxin (toxoid) antigens; 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 Concentrate)*: 1 bottle containing 50 ml of a 20-fold concentrated buffer for washing the wells; pH 7.2 ± 0.2; white cap.
- C. diphtheriae toxin anti-IgG Conjugate**: 1 bottle containing 20 ml of peroxidase labelled 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.
- C. diphtheriae toxin IgG Standards***: 5 vials each containing 2 ml; colored yellow; ready to use.
  - Standard A: 0.000 IU/ml; blue cap
  - Standard B: 0.015 IU/ml; green cap
  - Standard C: 0.075 IU/ml; yellow cap
  - Standard D: 0.150 IU/ml; red cap
  - Standard E: 1.000 IU/ml; white cap
• Strip holder
• 1 Cover foil
• 1 test protocol
• 1 distribution and identification plan.

* 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 (20-25 °C) before starting the test run.

2. **Coated snap-off Strips:** The ready to use break-apart snap-off strips are coated with C. diphtheriae toxin (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; stability until expiry date.*

3. **C. diphtheriae 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 stability until expiry date when stored at 2-8 °C.*

4. **Standards:** The vials labelled with Standard A, B, C, D and E contain a ready to use standard solution. The concentration of the standards, calibrated in accordance with the “International Standard for Diphtheria Antitoxin (00/496)”, are:
   - Standard A: 0.000 IU/ml
   - Standard B: 0.015 IU/ml
   - Standard C: 0.075 IU/ml
   - Standard D: 0.150 IU/ml
   - Standard E: 1.000 IU/ml
The solutions have to be stored at 2-8 °C and contain 0.1 % Kathon. 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 has to 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 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 stability until expiry date when stored at 2-8 °C.

8. **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 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 10 µl 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 E (1.00 IU/ml) a second 1 + 1 dilution of this 1 + 100 diluted patient 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 300 µl to 350 µl 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
- 5 well (e.g. B1, C1 etc.) for Standard A, B, C, D and E.

- **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 °C ± 1 °C.
Assay Procedure

1. Dispense 100 µl of each Standard (A, B, C, D and E) and diluted sample 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 100 µl C. diphtheriae 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.** *Do not expose to direct sunlight.*
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20-25 °C) in the dark.**
10. Dispense 100 µl 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. Dilute the specimen as mentioned under Sample Dilution.

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 standard 0.

*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. 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 > 0.050
- **Standard C** in D1: Absorbance > 0.200
- **Standard D** in E1: Absorbance > 0.400
- **Standard D** in F1: Absorbance > 1.000

Standard A < Standard B < Standard C < Standard D < Standard E

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 5 Standards A, B, C, D and E on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0.000, 0.015, 0.075, 0.150, 1.000)
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 patient 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 protective antibody level
- 0.01 - 0.09: No reliable protection
- 0.1 – 1.0: Reliable protection
- > 1.0: Reliable long term protection

E. Precision

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean value (NTU)</th>
<th>Cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak pos.</td>
<td>24</td>
<td>0.95</td>
<td>12.1</td>
</tr>
<tr>
<td>Pos.</td>
<td>24</td>
<td>3.02</td>
<td>2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean (IU/ml)</th>
<th>Cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-assay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak pos.</td>
<td>12</td>
<td>0.04</td>
<td>11.4</td>
</tr>
<tr>
<td>Pos.</td>
<td>12</td>
<td>0.16</td>
<td>2.8</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 84.6 %.

G. **Sensitivity**

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100 %.

H. **Analytical Sensitivity**

The analytical sensitivity – defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator – is 0.01 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>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></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Large CV</th>
<th>Bubbles in wells</th>
<th>Ensure no bubbles present prior to reading plate</th>
</tr>
</thead>
<tbody>
<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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low sensitivity</th>
<th>Improper storage of ELISA kit</th>
<th>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</th>
</tr>
</thead>
<tbody>
<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>
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</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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