ab108718

*Chlamydia trachomatis*

IgA Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human IgA-class antibodies against *Chlamydia trachomatis* in Human serum and plasma.

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

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# Table of Contents

1. Introduction .......................... 2
2. Assay Summary ......................... 4
3. Kit Contents .......................... 5
4. Storage and Handling ................. 6
5. Additional Materials Required ....... 6
6. Preparation of Reagents ............... 7
7. Preparation and Collection of Specimen 7
8. Assay Method .......................... 8
9. Data Analysis .......................... 12
10. Limitations ........................... 15
11. Specificity ............................ 15
12. Troubleshooting ...................... 16
1. Introduction

ab108718, Chlamydia trachomatis IgA Human ELISA Kit is intended for the qualitative determination of IgA-class antibodies against Chlamydia trachomatis in Human serum and plasma. The qualitative immunoenzymatic determination of IgA-class antibodies against Chlamydia trachomatis is based on the ELISA technique.

Chlamydiae are nonmotile, Gram negative and obligatory intracellular growing bacteria which form characteristic inclusions within the cytoplasm of parasitized cells. They are easily visible in the light microscope. Three different Chlamydia species pathogenic for Humans are known: Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci, and one species only pathogenic for animals (C. pecorum). Chlamydia trachomatis is the most prevalent agent of sexually transmitted diseases worldwide (400-500 million cases) and the number of infections is constantly growing, during childbirth, causing conjunctivitis or pneumonia in newborns. Untreated cases of chlamydial infection can lead to chronic salpingitis, possibly resulting in ectopic pregnancy or infertility. In males, C. trachomatis is a major cause of non-gonococcal urethritis.

A severe problem in Chlamydia infections is the frequent asymptomatic insidious course which may result in the initiation of
chronic diseases. In many instances primary infections are not recognized and only the sequelae caused by ascended, persisting agents are diagnosed.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mechanism of Infection</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. trachomatis</em></td>
<td>Direct or sexual transmission: The primary site of infection usually is the mucous membrane of the eye or the urogenital tract</td>
<td>Lymphogranuloma venereum (LGV) Trachoma Inclusion conjunctivitis of neonates and adults; Cervicitis, salpingitis, urethritis, epididymitis, proctitis and pneumonia of newborns</td>
</tr>
<tr>
<td><em>C. pneumoniae</em></td>
<td>Infiltration of the mucous membrane of the respiratory tract</td>
<td>Respiratory diseases discussed: endocarditis, coronary heart diseases</td>
</tr>
<tr>
<td><em>C. psittaci</em></td>
<td>Inhalation of feces from infected birds; contact with infected avian viscera</td>
<td>Ornithosis (Psittacosis)</td>
</tr>
</tbody>
</table>

**Infection Diagnostics:**

**PCR:**   
**Microscopy:** Giemsa stain.  
**Serology:** Detection of antigens by ELISA  
Detection of antibodies by IF, EIA, ELISA
2. Assay Summary

Microtiter strip wells are precoated with *Chlamydia trachomatis* antigens to bind corresponding antibodies of the specimen. 100 µl of Samples and controls added to the wells and incubated at 37°C for 1 hour. Wells washed.

Add 100 µl horseradish peroxidase (HRP) labelled anti-Human IgA conjugate. This conjugate binds to the captured Chlamydia-specific antibodies. Incubated at RT for 30 min. Wells washed.

The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. Incubated at RT for 15 min. The intensity of this product is proportional to the amount of Chlamydia-specific IgA antibodies in the specimen.

Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell platereader.

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3. Kit Contents

- **Chlamydia trachomatis Coated Wells (IgA)**: 12 breakapart 8-well snap-off strips coated with *Chlamydia trachomatis* antigen; in resealable aluminium foil.

- **IgA Sample Diluent***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured 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.

- **Chlamydia trachomatis anti-IgA Conjugate**: 1 bottle containing 20 ml of peroxidase labelled rabbit antibody to Human IgA; coloured violet, ready to use; black cap.

- **TMB Substrate Solution**: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.

- **Chlamydia trachomatis IgA Positive Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.

- **Chlamydia trachomatis IgA Cut-off Control***: 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.

- **Chlamydia trachomatis IgA Negative Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.
• 1 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 even after opening if stored at 2-8 °C.

5. Additional Materials Required

• Distilled or deionized water
• Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl and 1.0 ml
• Disposable pipette tips
• Microtiter well reader capable of reading absorbance at 450/620 nm.
• Incubator 37°C
• Manual or automatic equipment for rinsing wells
• Disposable tubes
• Timer
• Vortex mixer, or equivalent
6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (20- 25°C) before use.

2. **Coated snap-off strips**: The ready to use breakapart snap-off strips are coated with *Chlamydia trachomatis* antigen. 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. **Washing solution (20xconc.)**: 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 the concentrate is stable until the expiry date.**

4. All other reagents are supplied ready to use.

7. Preparation and Collection of Specimen

1. 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 (-70 to -20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

2. Heat inactivation of samples is not recommended.

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

8. Assay Method

Procedural Notes:

- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.

- The following test procedure is only validated for manual procedure. 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.
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 and
1 well (e.g. E1) for the positive control.

It is recommended to determine controls and patient 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 control and sample.
- Adjust the incubator to 37°C ± 1°C.

**Assay Procedure:**

1. Select the required number of microtiter strips or wells and insert them into the holder.
2. Dispense 100 μl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
3. Cover wells with the foil supplied in the kit.
4. Incubate for 1 hour ± 5 min at 37°C ± 1°C.
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 μl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec.
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.*

6. Dispense 100 µl *Chlamydia trachomatis* anti-IgA Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.

7. Incubate for 30 min at room temperature. Do not expose to direct sunlight.

8. Repeat step 5.

9. Dispense 100 µl TMB Substrate Solution into all wells

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

11. 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 patient 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.*

12. Measure the absorbance of the specimen at 450/620nm 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 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.
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 – 1.30.
**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.

*Example: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 = 0.76 / 2 = 0.38
Cut-off = 0.38*

C. Interpretation of Results:

- Samples are considered POSITIVE if the absorbance value is higher than 10% over the cut-off.
ab108718 *Chlamydia trachomatis* IgA Human ELISA Kit

- Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative → 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:**

\[
\text{Patient (mean) absorbance value} \times 10 = \frac{[\text{Abcam-Units} = \text{NTU}]}{\text{Cut-off}}
\]

*Example: 1.216 \times 10 = 32 \text{ NTU} (\text{Abcam Units})

\[
\frac{0.38}{\text{Cut-off: 10 NTU}}
\]

Grey zone: 9-11 NTU

Negative: <9 NTU

Positive: >11 NTU
E. Precision

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean (NTU)</th>
<th>Cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inte-assay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos. Serum</td>
<td>12</td>
<td>24.3</td>
<td>7.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean (OD)</th>
<th>Cv (%)</th>
</tr>
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<tr>
<td><strong>Intra-assay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos. Serum</td>
<td>24</td>
<td>0.882</td>
<td>4.7</td>
</tr>
</tbody>
</table>

F. 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 97.3 %.

G. 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 >95 %.
10. Limitations

- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
- Diagnosis of an infectious disease should not be established on the basis of 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.
- The *Chlamydia trachomatis* antigen which is coated on the plates is comprised of elementary bodies.
- A cross reaction with *Chlamydia pneumoniae* cannot be excluded with sera containing antibodies to LPS and MOMP.

11. Specificity

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.
## 12. 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>
<tr>
<td>Issue Description</td>
<td>Possible Cause</td>
<td></td>
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<tr>
<td>--------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
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</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation &amp; optimal sample storage conditions (eg. minimize freeze/thaws cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wash wells as per protocol recommendations</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></td>
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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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Chlamydia trachomatis

IgA Human ELISA Kit

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