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# Product Information

## *Chlamydia Trachomatis* IgA ELISA Kit

Catalog Number: EA101059
Storage Temperature: 2 – 8°C

# Instruction for Use

**THIS KIT IS INTENDED FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

### 1 INTRODUCTION

*Chlamydiae* are non-motile, 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 are known to be pathogenic for humans: *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Chlamydia psittaci*, and only one species is 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. Rates in sexually active young people are commonly between 5 % and 10 % in Europe.

### 1.1 Intended Use

The **ORIGENE *Chlamydia trachomatis* IgA Enzyme Immunoassay Kit** provides materials for the **qualitative** and **semi-quantitative** determination of IgA-class antibodies to Chlamydia trachomatis in human serum and plasma.

### 2 PRINCIPLE OF THE TEST

The **ORIGENE *Chlamydia trachomatis* IgA ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Samples are diluted with S*ample Diluent* and additionally incubated with *IgG-RF-Sorbent* to eliminate competitive inhibition from specific IgG. This pretreatment avoids false negative results. Microtiter wells as a solid phase are coated with inactivated Elementary Bodies Chlamydia trachomatis antigen. **Diluted** specimens and **ready-for-use controls** are pipetted into these wells. During incubation Chlamydia trachomatis-specific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgA antibodies are dispensed into the wells. During a second incubation this anti-IgA conjugate binds specifically to IgA antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of Chlamydia trachomatis-specific IgA antibody in the specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

### 3 WARNINGS AND PRECAUTIONS

- This kit is for research use only. For professional use only.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.2 mol/L $H_2SO_4$. It may cause skin irritation and burns.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

## 4 REAGENTS

### 4.1 Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with inactivated Elementary Bodies Chlamydia trachomatis antigen.
   (incl. 1 strip holder and 1 cover foil)
2. ***Sample Diluent ****, 1 vial, 100 mL, ready to use,
   colored yellow; pH 7.2 ± 0.2.
3. ***IgG-RF-Sorbent*,*** 1 vial, 6.5 mL, ready to use,
   colored yellow;
   Contains anti-human IgG-class antibody.
4. ***Positive Control ****, 1 vial, 1.0 mL, ready to use;
   colored yellow, red cap.
5. ***Negative Control ****, 1 vial, 2.0 mL, ready to use;
   colored yellow, yellow cap.
6. ***Cut-off Control ****, 1 vial, 2.0 mL, ready to use;
   colored yellow, black cap.
7. ***Enzyme Conjugate ****, 1 vial, 20 mL, ready to use,
   colored red,
   antibody to human IgA conjugated to horseradish peroxidase.
8. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
9. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.2 mol/L $H_2SO_4$,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
10. ***Wash Solution ****, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1
    see “Preparation of Reagents“.

* Contain non-mercury preservative.

#### 4.1.1 Material required but not provided

- A microtiter plate calibrated reader (450/620nm ±10 nm)
- Calibrated variable precision micropipettes
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionized or (freshly) distilled water
- Timer
- Absorbent paper

### 4.2. Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for two months if stored as described above.

### 4.3 Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

***Wash Solution***

Dilute *Wash Solution* **1+19** (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2.
Consumption: ~ 5 mL per determination.
Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use.
*The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.*

**4.4 Disposal of the Kit**

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

**4.5 Damaged Test Kits**

In case of any severe damage to the test kit or components, ORIGENE has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

## 5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma can be used in this assay.
Do not use hemolytic, icteric or lipemic specimens.

**5.1 Specimen Collection**

**Serum:**

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

**Plasma:**

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

**5.2 Specimen Storage and Preparation**

Specimens should be capped and may be stored for up to 3 days at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time should be frozen only once at –20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

**5.3 Specimen Dilution**

Prior to assaying each specimen is first to be diluted with *Sample Diluent*. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with *IgG-RF-Sorbent.*

1. Dilute each specimen **1+50** with *Sample Diluent*;
   e.g. 10 µL of specimen + 0.5 mL of *Sample Diluent*. **Mix well.**
2. Dilute this prediluted sample **1+1** with *IgG-RF-Sorbent*
   e.g. 60 µL prediluted sample + 60 µL *IgG-RF-Sorbent*. **Mix well**
3. **Let stand for at least 15 minutes at room temperature, mix well or overnight at 2 °C – 8 °C and mix well again.**
4. Take 100 µL of these pretreated samples for the ELISA.

**Please note**: Controls are ready for use and must not be diluted!

## 6 ASSAY PROCEDURE

**6.1 General Remarks**

- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**

- Once the test has been started, all steps should be completed without interruption.
- Use new disposable plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- During 37°C incubation cover microtiter strips with foil to avoid evaporation.

**6.2 Test Procedure**

Prior to commencing the assay, dilute *Wash Solution*, prepare samples as described in point 5.3 and establish carefully the distribution and identification plan supplied in the kit for all specimens and controls.

1. 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 *Neg. Control*,
   2 wells (e.g. C1+D1) for the *Cut-off Control* and
   1 well (e.g. E1) for the *Pos. Control*.

It is left to the user to determine controls and samples in duplicate.

2. Dispense
   **100 µL** of *Neg. Control* into well B1
   **100 µL** of *Cut-off Control* into wells C1 and D1
   **100 µL** of *Pos. Control* into well E1 and
   **100 µL** of each diluted sample with new disposable tips into appropriate wells.
   Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37 °C**.

4. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted *Wash Solution* **(300 µL per well)**. Strike the wells sharply on absorbent paper to remove residual droplets.
   **Important note:**
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense **100 µL** *Enzyme Conjugate* into each well, except A1.

6. Incubate for **30 minutes at room temperature (20 °C to 25 °C).**
   *Do not expose to direct sun light!*

7. Briskly shake out the contents of the wells.

Rinse the wells **5 times** with diluted *Wash Solution* (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

8. Add **100 µL** of *Substrate Solution* into all wells.
9. Incubate for **exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark**.
10. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.
    Any blue color developed during the incubation turns into yellow.
    **Note:** Highly positive samples can cause dark precipitates of the chromogen!
11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the *Stop Solution*.

**6.3 Measurement**

**Adjust** the ELISA microplate or microstrip 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 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.

**7 CALCULATION OF RESULTS**

**7.1 Validation of the Test Run**

The test run may be considered valid provided the following criteria are met:

| | |
|---|---|
| **Substrate blank in A1:** | Absorbance value **lower than 0.100** |
| ***Neg. Control* in B1:** | Absorbance value **lower than 0.200** |
| ***Cut-off Control* in C1/D1 :** | Absorbance value **between 0.350 – 0.850** |
| ***Pos. Control* in E1:** | Absorbance value **between 0.650 – 3.000** |

**7.2 Calculation**

**Mean absorbance value of Cut-off Control [CO]**

Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in C1/D1).
***Example:*** *(0.44 + 0.46) ÷ 2 = 0.45 = CO*

**7.3 Interpretation**

**NEGATIVE** **Mean OD sample < OD CO -10%**
**GREY ZONE** **OD CO -10% ≤ Mean OD sample ≤ OD CO + 10%**
Repeat test 2-4 weeks later - with new samples
Results in the second test again in the grey zone ⇒ **NEGATIVE**
**POSITIVE** **Mean OD sample > OD CO +10%**

**7.3.1 Results in ORIGENE Units [OU]**

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{CO}} = \text{[ORIGENE Units = OU]}$$

Example: $\frac{1.580 \times 10}{0.45} = 35$ OU

**Interpretation of Results**

| | |
|---|---|
| **NEGATIVE:** | **<9 OU** |
| **CUT-OFF VALUE:** | **10 OU** |
| **GREY ZONE (equivocal):** | **9-11 OU** |
| **POSITIVE:** | **> 11 OU** |

## 8 ASSAY CHARACTERISTICS

### 8.1 Assay Dynamic Range

The range of the assay is between 0.45- 60 OU/mL.

### 8.2 Specificity of Antigen (Cross Reactivity)

For Chlamydia trachomatis a cross-reactivity could be expected with Chlamydia pneumonia and Chlamydia psittaci samples, due to their close relationship.

The ORIGENE Chlamydia trachomatis IgA ELISA shows no cross-reactivity to Chlamydia pneumonia IgA. (Evaluation done with 104 samples and controls in ORIGENE Chlamydia trachomatis IgA ELISA and Chlamydia pneumonia IgA from another manufacturer.) The cross-reactivity study to Chlamydia psittaci is in evaluation.

For the following parameters no cross reactivity is found: Adenovirus-6, Brucella abortus, Epstein Barr-Virus (VCA), Herpes simplex Virus 1+2, Measles Virus, Mumps Virus, Parvovirus B19, Rubella Virus, Toxoplasma gondii and Varicella zoster Virus. (In total 72 high positive and 11 positive serum samples are assayed.)

## 9 LIMITATIONS OF USE

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

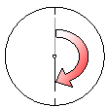
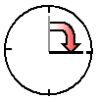
### 9.1 Interfering Substances

Hemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

## 10 REFERENCES


1. Weström L.V. (1996). Chlamydia and its effect on reproduction. J.Brit.Fertil.Soc. 1: 23-30.
2. Weström L. (1996). Consequences of genital Chlamydia infections in women. In: Chlamydia Research. Angelika Stary (ed.). Proceedings of the third meeting of the European Society for Chlamydia Research, Vienna, Austria, 11.-14. Sept. pp. 137-140.
3. Petersen E.E., Clad A. (1995). Genitale Chlamydieninfektionen. Deutsches Ärzteblatt 92, Heft 5, A-277-282.
4. Hoyme U.B., Spitzbart H. (1996). Past and current prevalence of Chlamydia trachomatis in women in Germany. In: Chlamydia Research. Angelika Stary (ed.). Proceedings of the third meeting of the European Society for Chlamydia Research, Vienna, Austria, 11.-14. Sept. p. 391.
5. Paavonen J. (1996). Chlamydia trachomatis: A major cause of mucopurulent cervicitis and pelvic inflammatory disease in women. In: Sexually Transmitted Diseases. Advances in Diagnosis amd Treatment. Curr. Probl. Dermatol. Elsner P., Eichmann A. (eds.), Basel, Karger, Vol. 24, pp. 110-122.
6. ECDC Guidance, Chlamydia control in Europe, Stockholm, June 2009.

## 11 SHORT INSTRUCTIONS FOR USE

| | |
|---|---|
|  | All reagents and specimens must be allowed to come to room temperature (18-25°C) before use. |
|  | Leave well A1 for substrate Blank.<br>Dispense 100 µL of Controls into appropriate wells. |
|  | Dispense 100 µL of sample into selected wells.<br>**(Please note special sample treatment, point 5.3!)** |
|   | Cover wells with foil.<br>Incubate for **60 minutes** at 37 °C. |
|  | Briskly shake out the contents of the wells. |
|  | Rinse the wells **5 times** with diluted Wash Solution<br>(300 µL per well). |
|  | Strike the wells sharply on absorbent paper to remove residual droplets. |
|  | Dispense 100 µL of Enzyme-Conjugate into each well. |
|   | Incubate for **30 minutes** at room temperature. |
|  | Briskly shake out the contents of the wells. |
|  | Rinse the wells **5 times** with diluted Wash Solution<br>(300 µL per well). |
|  | Strike the wells sharply on absorbent paper to remove residual droplets. |
|  | Add 100 µL of Substrate Solution to each well. |
|   | Incubate for **15 minutes** at room temperature. |

| | |
|---|---|
|  | Stop the reaction by adding 100 µL of Stop Solution to each well. |
|  | Determine the absorbance of each well at 450/620 nm. |

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