Version 25-05/24

**User's Manual**

# Chlamydia pneumoniae IgA ELISA Kit

REF **DEIA557A**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.


**Creative Diagnostics**

**Address: 45-1 Ramsey Road, Shirley, NY 11967, USA**

**Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)** **Fax: 1-631-938-8221**

**Email: info@creative-diagnostics.com** **Web: www.creative-diagnostics.com**

# PRODUCT INFORMATION

## Intended Use

The ELISA Chlamydia pneumoniae IgA tests are quantitative and qualitative immuoassays for detection of human antibodies in serum or plasma to Chlamydia pneumoniae.

## General Description

Chlamydiae are gram-negative intracellular bacteria. The following species cause disease in humans: Chlamydia trachomatis, Chlamydia pneumoniae, and Chlamydia psittaci. Chlamydia life cycle alternates between two distinct morphological forms: elementary bodies (EB) and reticular bodies (RB). The extracellular EBs are metabolically inert and are able to infect host cells where they transform into the metabolically active RBs. They multiply, recondense into EBS, and are released from the host cells to initiate another round of infection.

The different Chlamydia species have varying strategies to infect their human hosts and produce distinct diseases associated with each species. C. trachomatis is one of the most common sexually transmitted diseases, causing urethritis and cervicitis, but it may also, depending on the route of entry, result in conjunctivitis or pneumonia (newborn pneumonia). Ornithosis or psittacosis, resulting from infection with C. psittaci, manifests as a feverish pneumonia and may manifest systemically as myocarditis and/or endocarditis with possible fatal consequences. Infection with C. pneumoniae is generally asymptomatic or produces mild general symptoms such as pharyngitis, hoarseness, bronchitis, and coughing. Symptoms can range from acute pharyngitis, sinusitis, and bronchitis to pneumonia, myocarditis and endocarditis. Occasionally immunopathological syndromes can result, such as Erythema nodosum, arthralgia, Guillain-Barré-Syndrome, and myalgia. In addition, chronic infection with C. pneumoniae has been associated with asthma, COPD, arteriosclerosis, and cardiovascular disease. It is possible that C. pneumoniae is responsible for disease onset, leaving an infected individual susceptible to other pathogens, such as Streptococcus pneumoniae.

## Principles of Testing

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay which is particularly suited to the detection of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the EISA microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies are present in a sample, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the antigen-antibody complexes. The colorless substrate p-nitrophenylphosphate is then converted into a colored product p-nitrophenol. The signal intensity of this reaction product is proporational to the concentration of the antibody in the sample and is measured photometrically.

## Reagents And Materials Provided

**1. Break apart microtiter test strips each with eight antigen coated single wells,** (altogether 96), 1 frame. The coating material is inactivated. 12 pieces

**2. Standard serum (ready-to-use),** Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen), and anti-HCV Ab; preservative: <0.1% sodium azide;

coloring: Amaranth O. 2 × 2ml.

**3. Negative control serum (ready-to-use),** Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen), and anti-HCV Ab; preservative: <0.1% sodium azide; coloring: Lissamin Green V. 2ml

**4. Anti-human IgA conjugate (ready-to-use),** Anti-human IgA polyclonal antibody, Conjugated to alkaline phosphatase, stabilized with protein stabilization solution; preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane. 13ml

**5. Washing solution concentrate (sufficient for 1000ml),** Sodium chloride solution with Tween 20 and 30mM Tris/HCl, pH 7.4; preservative: <0.1% sodium azide. 33.3ml

**6. Dilution buffer (ready-to-use),** Protein-containing phosphate buffer with Tween 20; preservative: <0.1% sodium azide; coloring: 0.01g/l bromphenol blue, 2 × 50ml

**7. Stopping solution (ready-to-use),** <0.1N sodium hydroxide, 40mM EDTA. 15ml

**8. Substrate (ready-to-use),** Para-nitrophenylphosphate in solvent-free buffer; preservative: <0.1% sodium azide. 13ml

## Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtiter plates with filter, wavelength 405nm, recommended reference wavelength 620nm-690nm (e.g. 650nm)
3. Microtiter plate washer
4. Incubator 37°C
5. Moist chamber
6. Distilled water
7. Optional: ELISA control

## Storage

| Reagent | Storage | Stability |
|---|---|---|
| Microtiter strips (coated with antigen) | Unopened<br><br>After opening at 2-8°C in closed aluminum bag with desiccant | See expiry date<br><br>Minimum shelf life: four weeks |
| Control sera/ Standard sera | Unopened / after opening at 2-8°C | See expiry date |
| Conjugate | Unopened / after opening at 2-8°C | See expiry date |
| Dilution buffer | Unopened / after opening at 2-8°C | See expiry date |
| Washing solution | Unopened / after opening at 2-8°C<br><br>Working dilution at 2-8oC<br><br>Working dilution at room temperature | See expiry date<br><br>2 weeks<br><br>1 week |
| Substrate | Unopened / after opening at 2-8°C | See expiry date |
| Stopping solution | Unopened / after opening at 2-8°C | See expiry date |

## Specimen Collection And Preparation

### 1. Sample preparation and Storage

Lipemic, hemolytic, or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

### 2. Dilution of Samples

Before running the test, samples ($V_1$) must be diluted in dilution buffer ($V_2$) as follows:

| | | | |
|---|---|---|---|
| $V_1 + V_2 = 1:500$ | add | 10ul | sample |
| | up to | 1000ul | dilution buffer (= 1:100) |
| | add | 50ul | of the first dilution |
| | plus | 200ul | dilution buffer (= 1:5) |

### 3. Sample Storage

Samples should not be stored for more than 7 days at 2-8°C. Extended storage is possible at ≤ -20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

## Reagent Preparation

Bring all reagents to room temperature before use.

### 1. Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

### 2. Control Sera / Standard Sera (ready-to-use)

Control and standard sera are ready-to-use and must not be diluted further. For each test run – independent of the number of microtiter test strips to be used – control and standard sera must be included. Standard and cut-off sera should be set up in duplicate. Do not treat control sera with Rf-absorbent.

### 3. Anti-human IgA AP-Conjugate (ready-to-use)

The required conjugate concentration (+, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label.

### 4. Washing Solution (Concentrate)

Dilute washing buffer concentrate ($V_1$) 1:30 with distilled water to a final volume of $V_2$.

Example:

| Buffer concentrate ($V_1$) | Final volume ($V_2$) |
|---|---|
| 33.3ml | 1000ml |
| 1.0ml | 30ml |

**5. Dilution Buffer for Samples (ready-to-use)**

**6. Substrate (ready-to-use)**

Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product!

**7. Stopping Solution (ready-to-use)**

## Assay Procedure

1. Place the required number of wells in the frame and prepare a protocol sheet.
2. Add each 100ul of diluted sample or ready-to-use controls into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

| Well | Quantitative ELISA |
|---|---|
| A1 | Substrate blank |
| B1 | Negative control |
| C1 | Standard serum |
| D1 | Standard serum |
| E1 | Sample 1 . . . |
| F1 | Sample 2 . . . |

3. Sample incubation for 60 minutes (+/- 5 min.) at 37°C (+/- 1°C) in moist chamber.
4. After incubation wash all wells with washing solution (by automated washer or manually):

   aspirate or shake out the incubation solution

   fill each well with 300ul washing solution

   aspirate or shake out the washing solution

   repeat the washing procedure 3 times (altogether 4 times!)

   dry by tapping the microtiter plate on a paper towel
5. Addition of conjugate: Add 100ul of the ready-to-use IgA conjugate to the appropriate wells (except substrate blank).
6. Conjugate incubation for 30 minutes (+/- 1 min.) at 37°C (+/- 1°C) in moist chamber.
7. After incubation wash all wells with washing solution (see above).
8. Addition of substrate, Add 100ul of ready-to-use substrate solution to each well (including well for substrate blank!).
9. Substrate incubation for 30 minutes (+/- 1 min.) at 37°C (+/- 1°C) in moist chamber.
10. Stopping of the reaction, Add 100ul stopping solution to each well, shake microtiter plate gently to mix.
11. Read extinction, Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

## Quality Control

For the periodic verification of the test method, in order to fulfill the requirements of laboratory internal quality management systems, we recommend using ELISA controls to determine precision and accuracy of ELISA test runs. The use of ELISA controls is described in specific instruction manuals.

## Calculation

The mathematical curve fitting for antibody quantification with ELISA immunoassays is based on the 4-parameter logistic (4PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

Parameter A: Lower asymptote (OD)

Parameter B: Slope of the curve

Parameter C: Inflection point

Parameter D: Upper asymptote (OD)

CD establishes a lot-specific 4PL standard curve for each ELISA immunoassay in multiple test runs under optimal test conditions. The four parameters are indicated on the quality control certificate of each individual ELISA test.

For the adaptation of the test level to the given 4PL standard curve, the correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, and consequently test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ STD\ OD\ value}$$

By multiplying the OD values obtained from samples with the correction factor F, the level of each individual test run is adjusted to the given 4PL standard curve. Thereby, interassay deviations are compensated for and antibody activities can be directly evaluated from the 4PL standard curve.

After subtraction of the substrate blank from all measured OD values and calculation of the mean OD value of the standard serum (STD), tested in duplicate, a range of possibilities are available for the evaluation of antibody activities from the optical measurement signals (OD) of samples. They are described in separate manuals.

**Borderline Ranges**

The borderline ranges of the ELISA Chlamydia pneumoniae IgA tests are specified on the quality control certificates and indicate the range of borderline test results. Values below this range indicate a negative value; values above the borderline range indicate a positive value.

**Limits of Quantification**

The limits of quantification are specified on the quality control certificate of the ELISA classic Chlamydia

pneumoniae IgA. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. In case a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

**Criteria of Validity**

a. The substrate blank must be <0.25 OD.

b. The negative control must be negative.

c. By use of quantitative ELISA tests, the mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which given on the lot-specific quality control certificate.

d. By use of qualitative ELISA tests, the OD value of the positive control and the mean OD value of the cut-off serum must be within the validity ranges which are given on the lot-specific quality control certificate of the kit (after subtraction of the substrate blank!).

e. The variation of OD values of the standard serum or cut-off serum must not be higher than 20%.

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

## Interferences

**Evidence of Deterioration**

Optimum results can only be achieved if the instructions are strictly followed. Only use ELISA classic reagents when using ELISA immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of ELISA immunoassays are defined exclusively for the test kit to be used and

must not be used in other lots.

Each ELISA test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM), indicated on the label as + (low), ++ (medium), and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used of other ELISA immunoassays irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the tubes containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures.

Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate, or substrate can result in different preincubation times which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.

Adequate washing avoids non-specific reagent binding. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure, ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix up the caps of the bottles and/or vials.

The ELISA immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

**Statements of Warning**

The ELISA is designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and samples should be handled carefully using established good laboratory practice.

1. This kit contains human blood components. Although all control and cut-off sera have been tested and found negative for anti-HIV ab, HBs-Ag (Hepatitis B-Virus surface Antigen), and anti-HCV ab, they should be considered potentially infectious.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas in which samples or kit reagents are handled.
4. Wear disposable gloves, laboratory coat, and safety glasses while handling kit reagents or samples. Wash hand thoroughly afterwards.
5. Samples and other potentially infectious material should be decontaminated after the test run.
6. Reagents should be stored safely and be inaccessible to unauthorized access, e.g. children.