



Human Chlamydia pneumoniae IgA antibody ELISA Kit

Enzyme Immunoassay for the determination of Human Chlamydia pneumoniae IgA antibody in serum and plasma

Catalog number: ARG80530

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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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 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. Pregnant women infected with *C. trachomatis* may transmit these bacteria 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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A specific Chlamydia pneumoniae antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Chlamydia pneumoniae antibody present is bound by the immobilized antigen. After washing away any unbound substances, an HRP-conjugated antibody specific

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for human IgA is added to each well and incubate. Following the washing of any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of antigen-antibody binding in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm \pm 2nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Calibrator A (Negative Control)	2ml	4°C
Calibrator B (Cut-off Standard)	3ml	4°C
Calibrator C (Weak Positive Control)	2ml	4°C
HRP-conjugated antibody	20ml (Ready-to-use)	4°C
Sample Diluent	100ml	4°C
20X Wash buffer	50ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using citrate, heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. For the performance of the test, the samples have to be diluted 1:101 with sample diluent.

REAGENT PREPARATION

- **1X Wash buffer:** Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- **Samples:** Samples have to be diluted 1:100 with sample diluent.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of controls, diluted samples (1:100) and zero controls (sample diluent buffer) into wells. Incubate for 1h at 37°C.
3. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl HRP-conjugated antibody (ready-to-use) into each well. Cover wells and incubate for 30 minutes at RT.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature.
7. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

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

Substrate blank: Absorbance value <0.1

Negative control: Absorbance value <0.2 and $<\text{cut-off}$

Cut-off control: Absorbance value $0.15-1.3$

Positive control: Absorbance value $>\text{cut-off}$

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

2. Interpretation of results:

The samples are considered positive if the absorbance value is higher than 10% over the cut-off.

Samples with absorbance value of 10% above or below cut-off should be considered in the grey zone.

It is recommended to repeat test again 2-4 weeks later with fresh sample.

If the 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.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay precision is 4.9-8.3 % and inter-assay precision is 9.7-14%.