Creative Diagnostics®

Version 08-03/25

**User's Manual**

# Chlamydia Trachomatis IgG ELISA Kit

REF **DEIA-CL034**

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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.


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# PRODUCT INFORMATION

## Intended Use

The Chlamydia Trachomatis IgG ELISA Kit is intended for the detection of IgG antibody to Chlamydia Trachomatis in human serum or plasma.

## 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 ELISA 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 8 antigen coated single wells (altogether 96), 1 frame, the coating material is inactivated, 12
2. Standard serum (ready-to-use), Human serum in phosphate buffer with protein; negative for antiHIV-Ab, anti-HBs-Ag (Hepatitis B-Virus-surface antigen) and anti HCV-Ab; preservative: < 0.1 % sodium azide, coloring: Amaranth O. 2 × 2 ml
3. Negative control serum (ready-to-use), Human serum in phosphate buffer with protein; negative for anti-HIV, anti-HBs (Hepatitis B-Virus-surface antigen) and anti-HCV; preservative: < 0.1 % sodium azide, coloring: Lissamin green V . 2 ml
4. Anti-human-IgG-conjugate (ready-to-use), Anti-human-IgG from goat (polyclonal), conjugated to alkaline phosphatase, stabilized with protein stabilization solution preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane.13 ml.
5. Washing solution concentrate (sufficient for 1 litre), Sodium chloride solution with Tween 20, 30 mM Tris, preservative: < 0.1 % sodium azide. 33.3 ml
6. Dilution buffer, Phosphate buffer with protein and Tween 20; preservative: < 0.1 % sodium azide, 0.01 g/l Bromphenol blue sodium salt. 2 × 50 ml
7. Stopping solution, 1.2 N sodium hydroxide. 15 ml
8. Substrate (ready-to-use), Para-nitrophenylphosphate, solvent free buffer, preservative: < 0.1 % sodium azide. (Substrate in unopened bottle may have a slightly yellow coloring. This does not reduce the quality of the product!) 13 ml

## Materials Required But Not Supplied

1. common laboratory equipment

2. photometer for microtiter plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm- 690 nm (e.g. 650 nm)
3. incubator 37°C
4. moist chamber
5. distilled water

## Assay Procedure

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

   well A1: substrate blank

   well B1: negative control

   well C1: standard serum

   well D1: standard serum

   well E1: sample 1....
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 300 µl washing solution aspirate or shake out the washing buffer repeat the washing procedure 3 times (altogether 4 times!) dry by tapping the microtest plate on a paper towel
5. Addition of conjugate: Add 100 µl of IgG-conjugate (ready-to-use) to the appropriate well (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 100 µl substrate solution (ready-to-use) 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 100 µl stopping solution to each well, shake microtest plate gently to mix.
11. Read optical density: Read OD within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

* Please note, that under special working-conditions internal laboratory adaptations of the incubation times could be necessary.