DRG® Ureaplasma urealyticum IgG Elisa (EIA-4623)

REVISED 15 MAY 2011 RM (VERS. 7.1) **RUO IN THE USA**

THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

THIS KIT IS NOT INTENDED FOR DIAGNOSTIC PURPOSES.

1 INTRODUCTION

The **DRG Ureaplasma urealyticum IgG Enzyme Immunoassay Kit** provides materials for determination of IgG-class antibodies to Ureaplasma urealyticum in serum.

2 PRINCIPLE OF THE TEST

The **DRG Ureaplasma urealyticum IgG ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA)

Microtiter wells as a solid phase are coated with Ureaplasma urealyticum antigen.
Diluted sample specimens and **ready-for-use controls** are pipetted into these wells. During incubation Ureaplasma urealyticum-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 IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG 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 Ureaplasma urealyticum-specific IgG antibody in the specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

3 PRECAUTIONS

- **Please use only the valid version of the package insert provided with the kit.**
- 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.
- Controls and Standards has been found to be non-infectious in cell cultures.
- Avoid contact with *Stop Solution* containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
- 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.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

4 KIT COMPONENTS

4.1 Contents of the Kit

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with Ureaplasma urealyticum 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. ***Pos. Control*** ***, 1 vial, 1.0 mL, ready to use;
   colored yellow, red cap.
4. ***Neg. Control*** ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, yellow cap.
5. ***Cut-off Control*** ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, black cap.
6. ***Enzyme Conjugate*** **, 1 vial, 20 mL, ready to use,
   colored red,
   antibody to human IgG conjugated to horseradish peroxidase.
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
8. ***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.
9. ***Wash Solution*** *, 1 vial, 30 mL (20X concentrated for 600 mL), pH 7.2 ± 0.2
   see „Preparation of Reagents“.

* contains 0.03 % ProClin 300
** contains 0.03 % ProClin 300 + 0.01 % Gentamicin sulphate
*** contains 0.03 % ProClin 300 + 0.015 % 5-bromo-5-nitro-1,3-dioxane (BND) + 0.010 % 2-methyl-2H-isothiazol-3-one (MIT)

4.1.1 Equipment and material required but not provided

- A microtiter plate calibrated reader (450/620nm ±10 nm)
  (e.g. the DRG Instruments Microtiter Plate Reader)
- Calibrated variable precision micropipettes
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Timer
- Absorbent paper

4.2 Storage and stability of the Kit

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 four months if stored as described above.

4.3 Preparation of Reagents

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.
Consumption: ~ 5 mL per determination.
Crystals in the solution disappear by warming up to 37 °C in a water bath.
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 (see chapter 13 of this data sheet).

4.5 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG 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

Serum can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Specimens containing anticoagulant may require increased clotting time.

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 24 hours 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 dilute each specimen **1+100** with *Sample Diluent*;
e.g. 10 µL of specimen + 1 mL of *Sample Diluent,* **mix well, let stand for 15 minutes, mix well again.**

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

6 TEST PROCEDURE

6.1 General Remarks

- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- **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 disposal 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.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- During incubation cover microtiter strips with foil to avoid evaporation.

6.2 Assay 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. Cover wells with foil. Incubate for **30 minutes at 37 °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. Cover wells with foil. 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 RESULTS

7.1 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*

7.2 Interpretation

POSITIVE Sample (mean) absorbance values more than 10 % above CO
(Mean OD $_{Sample}$ > 1.1 x CO)

GREY ZONE Sample (mean) absorbance values from 10 % above to 10 % below CO
repeat test 2 - 4 weeks later - with new Sample samples
(0.9 x CO ≤ Mean OD $_{Sample}$ ≤ 1.1 x CO)

Results in the second test again in the grey zone ⇒ **NEGATIVE**

NEGATIVE Sample (mean) absorbance values more than 10 % below CO
(Mean OD $_{Sample}$ < 0.9 x CO)

7.2.1 Results in DRG Units [DU]

$$\frac{\text{Sample (mean) absorbance value x 10}}{\text{CO}} = \text{[DRG Units = DU]}$$

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

8 QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
If the results of the assay do not fit to the established acceptable ranges of control materials Sample results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 LIMITATIONS OF USE

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

10 LEGAL ASPECTS

10.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

10.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11 REFERENCES

Ureaplasma urealyticum (human mucous membranes) J.I. Glass et al., Nature (2000) 407:757-62

Measurement of Antibody to *Ureaplasma urealyticum* by an Enzyme-Linked Immunosorbent Assay and Detection of Antibody Responses in Patients with Nongonococcal Urethritis
Mary B. Brown, Gail H. Cassell, David Taylor-Robinson, and Maurice C. Shepard J Clin Microbiol. 1983 Febr; 17(2):288-295

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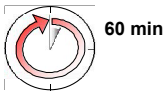
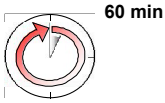
Serological characterisation of Ureaplasma urealyticum strains by enzyme-linked immunosorbent assay (ELISA).
Turunen H, Leinikki P, Jansson E. J Clin Pathol 1982 Apr; 35(4):439-43

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. Dispense 100 µl of Controls into appropriate wells.
	Dispense 100 µl of sample into selected wells. **(Please note special sample treatment, point 5.3!)**
	Cover wells with foil. Incubate for **60 minutes at 37°C.**
	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.
	Dispense 100 µl of Enzyme-Conjugate into each well.
	Incubate for **30 minutes at 37°C.**
	Briskly shake out the contents of the wells.
	Rinse the wells **5 times** with diluted Wash Solution (300 µl per well).

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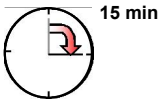
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Add 100 µl of Substrate Solution to each well.
	Incubate for **15 minutes** at room temperature.
	Stop the reaction by adding 100 µl of Stop Solution to each well.
	Determine the absorbance of each well at 450 nm.

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