DRG

DRG® CagA (EIA-4138)

CE

REVISED 7 MAR. 2011 RM (VERS. 3.1) **RUO IN THE USA**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Enzyme immunoassay for determination of Anti-CagA IgG antibodies in human serum or plasma.

1 PRINCIPLE OF THE ASSAY

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This kit is based upon an enzyme immunoassay method (ELISA), where horseradish peroxidase is used as enzyme conjugate. During the first incubation, the sample anti-CagA IgG antibodies, if any, are bound to the CagA antigen coated wells. A wash cycle eliminates all unbound material. In the incubation that follows, a second antibody (anti-human IgG conjugated with peroxidase) will bind to the CagA-antigen-antibody complex. After a further wash cycle a colorless Chromogen solution (tetramethylbenzidine, TMB) in a substrate-buffer is added to the wells, where it yields a colored compound, by reacting with the peroxidase enzyme. Color development will be stopped by adding H_2SO_4. The color intensity, measured in a spectrophotometer at 450 and 405 nm, will thus be directly proportional to the anti-CagA IgG antibody concentration in calibrators and samples.

2 REAGENTS PROVIDED WITH THE KIT: PREPARATION AND STABILITY

Reagents are sufficient for 96 tests.

Store the kit at 2-8°C.

The expiry date of each reagent is shown on the vial label.

Once opened, the kit is stable at 2-8°C for 2 months.

- **[MTP] Coated Microplate:**
  1 microplate for 96 breakable wells, coated with CagA antigen (obtained by recombinant DNA). Keep unused wells at 2-8°C in the provided plastic bag and accurately sealed.
- **[CAL] Calibrators:**
  6 vials (1.5 mL) of anti-CagA IgG in serum matrix at the following concentrations:
  0, 15, 30, 60, 120 and 240 RU/mL.
  Ready for use and red-colored. Preservative: NaN_3 (< 0.1%).
- **[CONJ] Enzyme Conjugate:**
  1 vial (14 mL) of mouse monoclonal anti-human IgG conjugated with horseradish peroxidase (HRPO) in TRIS buffer with stabilizers.
  Ready for use and pink-colored. Preservative: Neomycin.

- **[WASH] Washing Solution (concentrated):**
  1 vial (50 mL) of PBS-Tween 20. Preservative: Thimerosal (<0.05%).
  Just before use, dilute the needed amount 1:20 with distilled H_2O.
  In case of undissolved crystals, re-suspend the solution by placing the vial at 37°C for a few minutes.
  Store the diluted Washing Solution for 30 days at 2-8°C.
- **[DIL] Sample Diluent (concentrated):**
  1 vial (20 mL) of serum matrix and stabilizers, red-colored. Preservative: NaN_3 (<0.1%).
  Just before use, dilute 1:20 with the previously diluted Washing Solution.
  Store the diluted Sample Diluent for 30days at 2-8°C.
- **[TMB] Chromogen:** 2 vials (15 mL) of Tetramethylbenzidine (TMB) with citrate-phosphate buffer, DMSO and H_2O_2. Ready for use.
- **[STOP] Blocking Reagent:**
  1 vial (14 mL) of 1N H_2SO_4. Ready for use.
- **[CPA] Adhesive plate sealers**
- **Plastic bag**

3 MATERIAL REQUIRED BUT NOT SUPPLIED

3.1 Manual Test

- Adjustable, automatic micropipettes with disposable tips.
- Dry heater, adjustable at 37 ± 2°C.
- Graduated cylinders for reagent dilution.
- Aspiration pump or automated well washing device.
- Microplate spectrophotometer capable of measuring absorbances within a 0 - 3.0 A interval, at 450 nm and 405 nm.
- Millimetric graph paper.
- Distilled H_2O.

3.2 Automatic Test

This test can be used with automatic instrument for ELISA kits on microplate.

4 WARNINGS AND PRECAUTIONS

In order to obtain correct and reproducible results, the following rules must be observed:

- Do not mix reagents of different lots.
- Do not use reagents beyond their expiry date.
- Do not store or leave reagents and samples at high temperatures or areas of possible contamination.
- Use thoroughly clean glassware, free from metal ion contamination or oxidizing substances.

- Use distilled or deionized water, stored in perfectly clean containers.
- Carefully avoid any contamination among samples; for this purpose, disposable tips should be used for each sample and reagent.
- Do not modify in any way the "Assay Procedure". If you not respect:
  • exact incubation times and quantities adding the reagents
  • incubation times and temperature
  may cause incorrect clinical results.
- Reconstitute lyophilized reagents, if present, as described on the relative labels. Any deviation in reagent use or wrong volumes, may affect the reliability of results obtained.
- In case of manual procedure, it is important to use calibrated pipettes and have appropriate technical manuals. Primary importance is a good precision preparing and dispensing the reagents. Ensure that all the equipment used is in perfect working order, has been correctly calibrated and is regularly maintained.
- Ensure that the aspiration pump or automated well washing device is in perfect working order. Inadequate rinsing of wells may cause incorrect sample classifications. Ensure that all the equipment used is in perfect working order.
- Ensure that the microplate spectrophotometer is in perfect working order. The use of a not calibrated spectrophotometer or not clean filters may cause a wrong reading of samples with consequent incorrect samples classifications. Ensure that all the equipment used is in perfect working order.
- Ensure that the dry heater (if necessary) is in perfect working order. The incubation temperature different from 37 ± 2°C may cause a sensitivity losses and/or biological denaturation (samples and/or reagents). Ensure that the equipment used is in perfect working order and periodically check the recorded temperature.
- Ensure that the microplate shaker (if necessary) is in perfect working order. Incorrect agitation may cause wrong samples classifications. Ensure that the equipment used is in perfect working order.
- Ensure that all the equipment used for samples storage and/or the system is in perfect working order. The storage at different suggested temperature may cause biological material denaturation (samples and/or reagents). Ensure that the equipment used is in perfect working order and periodically check the recorded temperature.
- Use a suitable method for the correct identification of samples. Incorrect identification may cause specificity losses of the system and wrong clinical results.

In order to avoid personal and environmental contamination, the following precautions must be observed:

- Use disposable gloves while handling potentially infectious material and while performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- Chromogen and Blocking Reagent should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident rinse thoroughly with running water.
- All material of human origin (if any) used for preparing the reagents of this kit tested negative for HBsAg, anti-HIV and anti-HCV. No test however can currently grant total absence of such viruses. The above reagents as well as all human samples must be considered as potentially infectious.
- Avoid splashing and aerosol formation; in such cases, carefully wash with a 3% sodium hypochlorite solution. Any such cleaning material must be treated as potentially infectious and disposed of accordingly.
- Some reagents contain sodium azide as preservative; to prevent build-up of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.

- According to Italian decree D.L. no. 22 dated 05.02.97, in compliance with EEC directives (91/156/EEC, 91/689/EEC, 94/62/EEC), all waste products originating from either manual and/or automated processing are classified as hazardous special waste material (European classification code180103). As such, they must be eliminated by delegating to special enterprises, qualified for waste collection and disposal.

5 SPECIMEN COLLECTION AND PREPARATION

The assay can be performed in serum or plasma samples.
Moderately lipemic samples do not influence the results; highly lipemic or hemolyzed samples may affect the results. The presence of fibrin filaments could interfere with the assay; make sure that samples are always perfectly clear before testing. Keep samples properly stored at 2-8°C for 1 week; for longer periods it is advisable to freeze samples at -20°C. Repeated freezing and thawing of samples should be avoided.

Before use: Dilute samples **1:300** with previously diluted Sample Diluent (ex.10 µL sample + 2990 µL Diluent).

6 ASSAY PROCEDURE

Allow reagents and samples to warm up to room temperature.

Mix samples by inversion before use.

1. Prepare the wells for: Blank, Standards and Samples.
2. Pipette 100 µL of Standards and previously diluted Samples into the corresponding wells.
   Note: Standards must not be diluted.
3. Pipette 100 µL of Sample Diluent into the Blank well.
4. Cover the microplate with adhesive sheet (supplied with the kit) and incubate for 60±5 minutes at 37±2°C.
5. Wash the wells 4 times with 350 µL of diluted Washing Solution. Aspirate all liquid from the wells.
6. Add 100 µL of Enzyme Conjugate into all wells.
7. Cover the microplate with adhesive sheet (supplied with the kit) and incubate for 30±2 minutes at 37±2°C.
8. Wash the wells as described in point 5.
9. Pipette 100 µL of Chromogen into all wells.
10. Incubate for 15 minutes at 37±2°C. Avoid direct light exposure.
11. Dispense 100 µL of Blocking Reagent into each well.

12. Read the absorbance of the wells with a preferably bichromatic spectrophotometer at 450 nm, with reference wavelength at 620 nm (setting the instrument at zero with the Blank well). In case of overflow absorbance values, read at 405 nm. Reading must be completed within 15 minutes from the end of the assay.

(While using for the procedure an automatic instrument for microplates, refer to its relative manual.)

7 ASSAY SCHEME

Sample predilution: 1/300

Wells	Blank	Standard	Samples (dil.)
Reagents			
Standard	-----	100 µL	-----
Diluted Samples	-----	-----	100 µL
Sample Diluent	100 µL	-----	-----
Incubate: 37±2°C, 60±5 min.			
Aspirate and wash: 4 x 350 µL.			
Enzyme Conjugate	100 µL	100 µL	100 µL
Incubate: 37±2°C, 30±2 min.			
Aspirate and wash: 4 x 350 µL.			
Chromogen	100 µL	100 µL	100 µL
Incubate: 37°C, 15 min.			
Blocking Reagent	100 µL	100 µL	100 µL
Read: 450 nm.			

8 CALCULATION OF RESULTS

Draw a standard curve on linear graph paper, by plotting the standard concentrations (x-axis) against the absorbances obtained for each standard (y-axis). Corresponding anti-CagA IgG concentrations in RU/mL are obtained by interpolating the absorbance of each sample on the standard curve.

- Samples with IgG values **less than 10 RU/mL** must be considered as **non-reactive** for anti-CagA IgG antibodies.
- Samples with IgG values **within 10 to 15 RU/mL** must be considered as **weakly reactive**.
- Samples with IgG values **higher than 15 RU/mL** must be considered as **reactive** for anti-CagA IgG antibodies.

8.1 Example of Calculation

The following values must be taken as an example and should not be used in place of experimental values.

Description		Absorbance 450 nm	anti-CagA IgG
Standard	0 RU/mL	0.030	
Standard	15 RU/mL	0.225	
Standard	30 RU/mL	0.430	
Standard	60 RU/mL	0.815	
Standard	120 RU/mL	1.540	
Standard	240 RU/mL	2.210	
Sample		0.935	70 RU/mL

By interpolation on the standard curve, the resulting sample anti-CagA IgG titer was 70 RU/mL.

8.2 Validation Criteria

Before proceeding in calculating the results, make sure the control absorbances are within the following expected values:

Description	Expected values
Negative Control	< 0.100
OD Cal 240 RU/mL / OD Cal 15 RU/mL	> 2.5
OD Cal 15 RU/ mL / OD Cal 0 RU/mL	>3.5

If the values obtained are not as expected, it will be necessary to repeat the assay.

9 REFERENCES / LITERATURE

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Version 2011-02-24~rm