Version 19-04/24

User's Manual

Human Cardiolipin IgG ELISA Kit

REF **DEIA1708**

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

Enzyme immunoassay for the qualitative and quantitative determination of IgG antibodies against cardiolipin in human serum or plasma (EDTA, citrate, heparin).

General Description

The present enzyme-linked immuno sorbent assay (ELISA) is intended for the quantitative or qualitative determination of IgG antibodies directed against CL/β2-GP1 in human serum or plasma.

Principles of Testing

The immobilised antigen is a combination of CL, isolated from bovine heart, and a highly purified preparation of native β2-GP1. The test is fast (incubation time 30 - 30 - 30 minutes) and flexible (divisible solid phase, ready-to-use reagents). Six calibrators allow quantitative measurements; a negative and a positive control check the assay performance.

Reagents And Materials Provided

1. **Microtiter Plate**, coated with CL/β2-GP1 and hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells.
2. **Enzyme Conjugate IgG** ,14 mL, ready-to-use, red coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.
3. **Calibrator A-F**, 2 mL each, 0, 3, 8, 18, 45 and 100 GPL-U CL/β2-GP1 IgG / mL, ready-to-use, gradually blue coloured. Contain TBS, BSA, Tween and Na-azide.
4. **Negative and Positive Control**, 2 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.
5. **Sample Diluent**, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.
6. **TMB Substrate Solution**, 14 mL, ready-to-use, colourless. Contains a buffered solution of TMB and H_2O_2. Contained in a vial impermeable to light.
7. **Wash Buffer**, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.
8. **TMB Stop Solution** (0.2 M H_2SO_4), 14 mL, colourless, ready-to-use. Caution: sulfuric acid is corrosive.
9. Directions for use.
10. Lot-specific certificate of analysis.

Materials Required But Not Supplied

1. Deionised or distilled water
2. Graduated cylinder, 1000 mL

3. Tubes for sample dilution (transfer tubes in the microwell plate format recommended)
4. Pipettes for 10, 100 and 1000 µL (1- and 8-channel pipettes recommended)
5. Microwell plate washer (optional)
6. Microwell plate photometer fitted with a 450 nm filter
7. ELISA evaluation program (recommended)

Storage

Store kit at 2 - 8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Specimen Collection And Preparation

Preparation of the samples: handle specimens as potentially infectious agents. Besides serum, EDTA-, citrate- or heparin-treated plasma is suitable sample material as well. Specimen requirements: highly lipemic, haemolysed or microbially contaminated samples may cause erroneous results and should be avoided.

Prepare samples using normal laboratory techniques. Turbid samples must first be clarified (centrifuged). The clarified or clear samples are mixed and then diluted 1/100, e.g. 10 µL serum or plasma + 990 µL sample buffer. Also mix the dilution.

For rapid dispensing during the assay procedure, preparation of the calibrators, controls and samples in microwell transfer tubes is recommended. This allows the operation of an 8-channel pipette during the assay procedure. If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days. For longer storage, -20°C or lower temperatures are recommended. Repeated freezing and thawing of samples should be avoided. Thawed samples must be mixed prior to diluting.

Reagent Preparation

1. The solid phase must reach room temperature before opening the pouch. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.
2. Dilute the wash buffer 10x-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated (2 - 8°C).

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions. If the kit is to be used for several tests, only the currently needed amounts of reagents should be withdrawn. It is crucially important that no cross-contamination between the reagents occurs. Use only clean pipettes and do not pour back residues into the original flasks.

Assay Procedure

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C).

To achieve best results, i.e. the maximum ratio between specific and background signal, careful washing is essential (steps 1, 3 and 5). It is crucially important to remove the wash solution completely. For that purpose, tap the plate firmly on several layers of absorbent tissue. Automated washers must be verified according to

results obtained by manual washing.

1. Immediately prior to use, wash the solid phase once: fill wells with 350 µL wash buffer each, let soak for about 10 seconds in the wells and remove.
2. Dispense the calibrators (2.0 mL each, ready-to-use, gradually blue), controls (2.0 mL each, ready-touse, green and red) and the diluted samples rapidly into the microwells; 100 µL per well. Duplicate measurements are recommended. Incubate the plate for 30 minutes at room temperature (23 ± 3°C).
3. Wash the wells 4 times as in step 1.
4. Rapidly (preferably using an 8-channel pipette) dispense the conjugate (14 mL, ready-to-use, red); 100 µL per well. Incubate the plate as in step 2.
5. Repeat wash step 3.
6. Rapidly (preferably using an 8-channel pipette) dispense the substrate solution (14 mL, ready-to-use, colourless, black vial); 100 µL per well. Incubate the plate as in step 2. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.
7. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colourless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The colour changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.
8. Immediately read the absorbance in the microwell plate photometer at 450 nm.

Refrigerate the remainder of the reagents (2 - 8°C) if they are to be used again.

Quality Control

Quality control: the positive and negative control check the assay performance. Their authorised values and acceptable ranges, respectively, are quoted in the lot-specific certificate of analysis. Values of the controls must fall within the indicated ranges; otherwise, the results of the assay are invalidated.

Calculation

Quantitative evaluation: the data obtained are quantitatively evaluated with the standard curve. The curve has been constructed with a conventional ELISA evaluation program, using a 4-parameter function. The Spline approximation is also appropriate.

If no computer-supported evaluation is possible, the standard curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into GPL-U CL/β2-GP1 IgG per mL sample.

Qualitative evaluation: the test may also be evaluated in a qualitative manner. This requires measurement of the positive control only. Nevertheless, measurement and examination of the negative control is recommended (see below: quality control).

In qualitative test evaluation, the absorbance of the samples is compared with the borderline absorbance (= cut-off). It is determined according to the following formula:

Absorbance borderline = absorbance positive control x factor

The factor depends on the kit lot and is quoted in the lot-specific certificate of analysis which is included with each test kit.

Example:
absorbance positive control = 1250 mOD

factor = 0.35

absorbance borderline = 1250 mOD x 0.35 = 438 mOD

In order to gain an impression of how positive a particular sample is for CL/β2-GP1 IgG, one may calculate the ratio, according to the formula:

ratio = absorbance sample / absorbance borderline

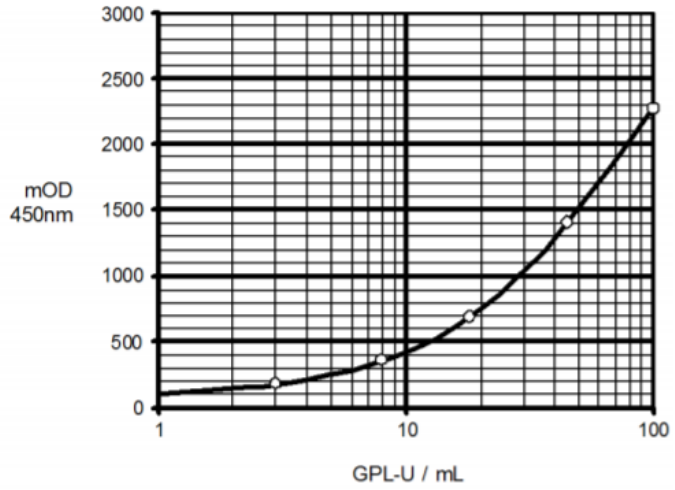
Example:
absorbance borderline = 438 mOD

absorbance sample = 1480 mOD

ratio = 1480 mOD / 438 mOD = 3.4

Typical Standard Curve

The depicted curve can only serve as a model. It can not substitute the measurement of the calibrators, together with the controls and actual samples.



Precision

For the assessment of the test precision, the variability of results under the following conditions wasdetermined: a. within 1 assay and between 3 assays, b. between 3 operators and c. between 2 kit lots.

a. Intra- and inter-assay variability (n = 24 and 72, respectively)

sample	mean GPL-U/mL	variability (cv, %) intra-assay	inter-assay
1	10	3,2	4,6
2	19	3,2	3,4
3	65	2,8	2,9

b. Operator to operator variability (n = 12)

sample	mean GPL-U/mL	variability (cv, %)
1	10	9,6
2	20	5,5
3	65	4,2

c. Variability between 2 kit lots (n = 6)

sample	mean GPL-U/mL	variability (cv, %)
1	11	4,6
2	21	2,5
3	73	3,1

Detection Range

2 - 100 GPL-U CL/β2-GP1 IgG per mL sample

Detection Limit

The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of Sample Diluent plus 3-fold standard deviation (s). It was determined as < 0.5 GPL-U CL/β2-GP1 IgG per mL sample

Sensitivity

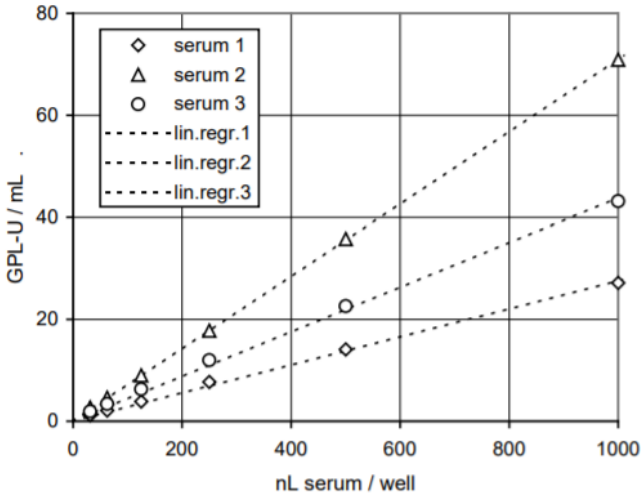
It was determined as < 0.5 GPL-U CL/β2-GP1 IgG per mL sample

Specificity

The test allows the specific determination of human IgG antibodies directed against CL/β2-GP1.

Linearity

In order to assess the dose-response relationship of the test, positive sera were measured in serial 2-fold dilution. Acceptance criterion: linear regression of 4 successive dilutions must yield a correlation factor>0.98. A typical result is depicted below.



Reproducibility

mOD-coefficient of variation (cv) over the plates < 8%.

Precautions

For research Use only. Not for use in diagnostic procedures. Not for internal or external use in humans or animals. It must be executed by trained personnel staff.

Do not use reagents beyond their expiration dates.

Adherence to the protocol is strongly recommended.

The Sample Diluent, calibrators and controls contain Na-azide as antimicrobial agent. The wash buffer contains bromonitrodioxane and the conjugate methylisothiazolone / bromonitrodioxane as preservative.

The substrate contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2). The stop solution, 0.2 M sulfuric acid (H_2SO_4), is acidic and corrosive.

The above mentioned reagents may be toxic if ingested. Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact with skin or mucous membrane, wash thoroughly with water. Never pipette by mouth. Dispose in a

manner complying with local/national regulations.

Na-Azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.

The calibrators and controls contain components of human origin. They were tested for human immunodeficiency virus (HIV)-Ag, hepatitis B surface (HBs)-Ag and antibodies against HIV 1/2 and hepatitis C virus (HCV) and showed negative results; either in an FDA-approved or a CE-compliant test, according to European Directive 98/79/EC.

However, no test can guarantee that material of human origin is not actually infectious. The preparations should therefore be treated as potentially infectious and disposed of accordingly, as should the samples (and residues thereof); according to CDC (Center of Diseae Control, Atlanta, USA) or other local / national guidelines on laboratory safety and decontamination.