Version 16-04/24

User's Manual

Human Anti-Cardiolipin Antibody IgA ELISA kit

REF **DEIA-BJ895**

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

Anti-Cardiolipin IgA ELISA is a test system for the quantitative measurement of IgA class autoantibodies against cardiolipin in human serum or plasma.

Principles of Testing

Highly purified cardiolipin is coated on microwells saturated with beta-2-glycoprotein I.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue colored product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

Reagents And Materials Provided

Provided materials	Format	
Microplate	1	One divisible **microplate** consisting of 12 modules of 8 wells each. Ready to use.
Calibrator A	1 x 1.5 ml	**0 APL-U/mL**, containing serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
Calibrator B	1 x 1.5 ml	**7.5 APL-U/mL**, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
Calibrator C	1 x 1.5 ml	**15 APL-U/mL**, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
Calibrator D	1 x 1.5 ml	**30 APL-U/mL**, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
Calibrator E	1 x 1.5 ml	**60 APL-U/mL**, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, NaN_3 0.09%), yellow. Ready to use.
Calibrator F	1 x 1.5 ml	**120 APL-U/mL**, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, NaN_3 0.09%), yellow. Ready to use.
Control Positive	1 x 1.5 ml	Containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
Control Negative	1 x 1.5 ml	Containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
Sample Buffer P	20 ml	Containing PBS, BSA, detergent, preservative NaN3 0.09%, yellow, concentrate **(5 x)**.
Enzyme Conjugate	15 ml	Containing anti-human **IgA** antibodies, HRP labelled; PBS, BSA, detergent, preservative Proclin 0.05%, light red. Ready to use.
TMB Substrate	20 ml	Containing 3,3', 5,5'-Tetramethylbenzidin, colorless. Ready to use.
Stop Solution	1	Contains acid. Ready to use.
Wash Buffer	1	Containing Tris, detergent, preservative NaN3 0.09%; 50X conc.
Instruction and Certificate of Analysis		

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm
2. Multi-Channel Dispenser or repeatable pipet for 100 µl
3. Vortex mixer
4. Pipets for 10 µl, 100 µl and 1000 µl
5. Laboratory timing device
6. Data reduction software
7. Distilled or deionized water
8. Graduated cylinder for 100 and 1000 ml
9. Plastic container for storage of the wash solution

Storage

• Store test kit at 2-8°C in the dark.

• Do not expose reagents to heat, sun, or strong light during storage and usage.

• Store microplate sealed and dessicated in the clip bag provided.

• Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.

• Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.

We recommend consumption on the same day.

Specimen Collection And Preparation

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

Assay Procedure

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots and products.
3. All materials must be at room temperature (20-28°C) prior to use.
4. Prepare all reagents and samples. Once started, performe the test without interruption.
5. Double determinations may be done. By this means pipetting errors may become obvious.
6. Perform the assay steps only in the order indicated.
7. Always use fresh sample dilutions.
8. Pipette all reagents and samples into the bottom of the wells.
9. To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
10. Wash microwells thoroughly and remove the last droplets of wash buffer.
11. All incubation steps must be accurately timed.
12. Do not re-use microplate wells.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted donor samples.
2. Pipet **100 µl** of calibrators, controls and prediluted donor samples in duplicate into the wells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D	P4										
E	E	P5										
F	F	P6										
G	C+	P7										
H	C-	P8										

P1: ...Sample, A-F: Calibrators, C+,C-: Controls

3. Incubate for 30 minutes at room temperature (20-28°C)
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature
7. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution
8. Dispense **100 µl** of TMB substrate solution into each well
9. Incubate for 15 minutes at room temperature
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed color is stable for at least 30 minutes. Read optical densities during this time.

Interpretation Of Results

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.

If these quality control criteria are not met the assay run is invalid and should be repeated.

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of samples may then be estimated from the calibration curve by interpolation.

Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

The assay system is calibrated against the internationally recognized reference sera from E.N. Harris, Louisville and the specific reference material IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

Negative: < 10 APL-U/ml

Positive: ≥ 10 APL-U/ml

Detection Range

0 - 120 APL-U/ml

Detection Limit

1 APL-U/ml

Interferences

No interference has been observed with hemolytic (up to 1000 mg/dL) or lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma.

Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin).

However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Precautions

1. All reagents of this kit are intended for professional use only.
2. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
4. Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
5. Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
6. Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
7. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
8. During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
9. Personal precautions, protective equipment and emergency procedures:

   • Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

   • Exposure controls/personal protection: Wear protective gloves of nitril rubber or natural latex.

   • Wear protective glasses. Used according to intended use no dangerous reactions known.
10. Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
11. For disposal of laboratory waste the national or regional legislation has to be observed.
12. Observe the guidelines for performing quality control in medical laboratories by assaying control sera.