



RELISA[®] CARDIOLIPIN ANTIBODY SCREENING TEST

For in vitro Diagnostic Use

For Professional Use

Catalog numbers: 7096-01 (96 wells) and 7696-01 (576 wells)

INTENDED USE: This is an enzyme immunoassay (EIA) test system for the qualitative detection of IgG, IgA, or IgM antibodies to cardiolipin in human serum. This test system is to be used as an aid in assessing the risk of thrombotic disorders in individuals with systemic lupus erythematosus or lupus-like syndromes.

SUMMARY AND EXPLANATION OF THE TEST

Antiphospholipid antibodies, including anticardiolipin antibodies, are frequently detected in sera from patients with systemic lupus erythematosus (1). Numerous reports have associated these autoantibodies with various venous and arterial thrombotic disorders, including cerebral infarction (2), deep venous thrombosis (3), thrombocytopenia (1), pulmonary embolism (4), and recurrent fetal loss with placental infarction (5). The “lupus anticoagulant” (6), a substance that prolongs the activated partial thromboplastin test *in vitro*, has also been associated with these clinical syndromes, although it is not identical to anticardiolipin antibody. The terms “lupus anticoagulant” and “antiphospholipid antibodies” are sometimes incorrectly used interchangeably, although these immunoglobulins are not the same (1).

The Immuno Concepts test system is a microwell enzyme immunoassay for detection of anticardiolipin antibodies in human serum. It has been shown previously that solid-phase immunoassay is an extremely sensitive and specific method for detection of anticardiolipin antibodies (7-10). The Immuno Concepts test system has been standardized using an internationally recognized reference preparation obtained from the Antiphospholipid Standardization Laboratory, (the “Harris Standards” (9)). The qualitative results obtained in this assay indicate the presence or absence of antibodies to cardiolipin.

PRINCIPLE OF THE TEST

This test is a qualitative indirect EIA. A stabilized preparation of cardiolipin has been coated onto the surface of the microwells to serve as an antigen in this system. Calibrator serum, controls, and diluted patient samples are placed in the microwells and incubated, allowing anticardiolipin antibodies in the sample to react with the antigen on the solid phase. After washing to remove unbound antibody and other serum proteins, the wells are incubated with goat anti-human antibodies that are labeled with horseradish peroxidase. The horseradish peroxidase-conjugated antibody preparation that is included in the test system will detect human IgG, IgA, and/or IgM, but will not differentiate between them. For the detection and semi-quantitation of specific IgG and IgM anticardiolipin antibodies, use the Immuno Concepts RELISA[®] Cardiolipin IgG & IgM Antibody Test System, catalog number 7096-02.

After incubation with horseradish peroxidase conjugate, if results are positive, a stable three-part complex is formed. This complex consists of horseradish peroxidase-conjugated anti-human antibody bound to human anticardiolipin antibody, which is bound to the cardiolipin stabilized on the plastic surface.

After another washing step, this complex is detected by adding a solution of tetramethylbenzidine (TMB) and H₂O₂ as a chromogenic substrate. The degree of color development in each well is proportional to the concentration of anticardiolipin antibodies in each serum sample. Each microwell is read in a spectrophotometer, and results are obtained by comparison of the absorbances of the calibrator wells and the absorbances of the sample wells.



SYSTEM COMPONENTS - MATERIALS PROVIDED

Storage: All components should be stored under refrigeration between 2-10°C. Do not freeze.

Stability: All components remain stable at least 12 months from date of manufacture. Do not use any component beyond its expiration date.

REACTIVE REAGENTS

Cardiolipin Coated Microwell Strips **PLATE**: Catalog number 7008-01. A microwell frame containing twelve eight well strips coated with a stabilized solution of diphosphatidylglycerol (cardiolipin) from beef heart. If fewer than eight wells are needed for testing, the wells can be separated by snapping them apart. The unused strips can be returned to the foil pouch with the desiccant pack, sealed with the zipper seal, and refrigerated for up to 45 days.



Sample Diluent **SOLN|DIL**: Catalog number 7100 (100 ml). Proprietary buffered sample diluent used to dilute patient samples. This diluent contains apolipoprotein H cofactor.



Anticardiolipin Antibody Positive Control Serum **CONTROL|+**: Catalog number 7021-01. Vial containing 2 ml of ready to use anticardiolipin positive human control serum. This serum contains IgG, IgM or IgA anticardiolipin antibodies. This serum shows strong positive color development in this assay.

Anticardiolipin Antibody Negative Control Serum **CONTROL|-**: Catalog number 7031-01. Vial containing 2 ml of ready to use anticardiolipin negative human control serum. This serum will not show color development in this assay.

Anticardiolipin Antibody Calibrator Serum **CAL**: Catalog number 7026-01. Vial containing 2 ml of ready to use liquid stable human anticardiolipin positive calibrator serum. This serum contains IgG and IgM anticardiolipin antibodies. This calibrator serum provides the cut-off value for this assay.

Enzyme Antibody Reagent - Human IgG, IgA, and IgM Specific **CONJ|HRP**: Catalog number 7009-01 (14 ml). Polyvalent anti-human IgG, IgM, and IgA conjugated to horseradish peroxidase (HRP). Reagent is ready to use.

Substrate Solution **SOLN|SUB**   : Catalog number 7035 (14 ml). HRP-specific enzyme substrate solution, containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂). Reagent is ready to use. **DANGER:** Flammable. This reagent contains less than 25% of methanol and acetone. Keep out of the reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician.

Stopping Reagent **SOLN|STOP**   : Catalog No. 7033 (14 ml). Proprietary stopping reagent for Immuno Concepts EIA test systems. Reagent is ready to use. **DANGER:** Corrosive. This reagent contains hydrochloric and sulfuric acids (less than 3% each, by volume), and should be handled with care. Keep out of the reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician. Never add water to this reagent.

NON-REACTIVE COMPONENTS

Holder for microwells

PBS Buffer **PWDR|PBS**: Catalog number 1011. Phosphate-buffered saline powder (0.01 M, pH 7.4 ± 0.2). Each pouch contains sufficient buffer powder to make one liter. Two pouches of buffer powder are supplied for each 96-microwell plate in complete test kits.

Preparation: Dissolve one pouch of buffer powder in one liter of deionized or distilled water and store between 2-25°C for up to 4 weeks or until signs of contamination or other visible changes occur. **DO NOT ADD TWEEN 20 OR OTHER DETERGENTS TO THIS BUFFER.**

ADDITIONAL MATERIALS REQUIRED - BUT NOT PROVIDED

Volumetric precision pipettors to deliver 10-1000 µl volumes
Squeeze bottle for delivering wash buffer solution to microwells, or an automated wash system for microwells
One-liter container for PBS wash buffer
Deionized or distilled water
Plate reading spectrophotometer capable of reading absorbance at 450 nm
Test tubes to prepare serum dilutions
Bibulous paper or paper towels
Multichannel pipettor capable of delivering to 8 wells
Disposable gloves
Lab timer

PRECAUTIONS

1. All human source materials used for this product have been tested and found negative (not repeatedly reactive) for antibodies to Human Immunodeficiency Virus-1 (HIV-1), Human Immunodeficiency Virus-2 (HIV-2), hepatitis C virus (HCV), and for hepatitis B surface antigen (HBsAg) by FDA approved methods. However, no test method can offer complete assurance that HIV-1, HIV-2, hepatitis C, hepatitis B, or other infectious agents are absent. Thus, all materials should be handled in the same manner as potentially infectious materials.
2. All control sera, calibrator sera, and patient samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual: *Biosafety in Microbiological and Biomedical Laboratories, 1999 Edition*.
3. Sodium azide (0.09%) is used as a preservative in the control and calibrator sera. Sodium azide may react with lead or copper plumbing and form highly explosive metal azides. When disposing of reagents, flush with ample volumes of tap water to prevent potential residues in plumbing. Sodium azide is a poison and may be toxic if ingested.
4. Dilution of the components or substitution of components other than those provided in this system may yield inconsistent results.
5. Do not heat inactivate serum samples to be used for anticardiolipin testing. Heat inactivation can cause elevated values.
6. This kit is for *in vitro* diagnostic use.
7. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
8. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
9. Avoid splashing or generation of aerosols at all times.
10. Incubation times and temperatures other than those specified may give erroneous results.
11. Cross contamination of reagents or samples may give false results. Samples must remain confined to microwells during testing.
12. Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use.
13. Bring all reagents, microwells, and specimens to room temperature (18-25°C) prior to use.
14. Wear disposable gloves when handling specimens and reagents, and wash hands thoroughly afterwards.
15. Microbial contamination of reagents or samples may give false results.
16. The stopping reagent is corrosive, and may cause burns. This reagent contains hydrochloric and sulfuric acids (less than 3% each, by volume), and should be handled with care. Keep out of the reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician. Never add water to this reagent.

SPECIMEN COLLECTION

Collection: Serum is the preferred specimen. Approximately 5 ml of whole blood should be collected aseptically by venipuncture using a sterile vacuum collection tube or other suitable collection system. Allow blood to clot at room temperature (18-25°C). Serum should be separated from the clot by centrifugation as soon as possible to minimize hemolysis. Immuno Concepts does not recommend the use of plasma in this assay because of the possibility of contamination of plasma with platelets. Platelets can affect the results by reacting with antiphospholipid antibodies.

CAUTION: Do not heat inactivate serum samples to be used for anticardiolipin testing. Heat inactivation can cause elevated values.

Interfering Substances: Sera exhibiting a high degree of hemolysis, icterus, lipemia, or microbial growth should not be used because these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Storage: Sera may be stored at 2-10°C up to one week. If testing is further delayed, sera should be stored frozen at -20°C or lower. Serum should not be stored in a self-defrosting freezer.

CAUTION: *Repeated freeze/thawing of patient samples may yield false positive or false negative results.*

GENERAL PROCEDURAL NOTES

1. It is extremely important to have all kit components and serum samples at room temperature (18-25°C) before use. A full liter of wash buffer may require several hours to warm to 20°C after removal from the refrigerator. Incubation temperatures above or below the stated range may cause inaccurate results. Return unused samples and reagents to refrigerated storage after use.
2. Mix reagents well before use by gentle inversion. Do not vortex or shake reagents. Avoid foaming.
3. When preparing sample dilutions, pipette tips should be wiped prior to dispensing serum into sample diluent. Excess sample adhering to the outside of the pipette tip will affect results.
4. The use of a multichannel pipettor is recommended because it provides more uniform reagent dispensing, incubation times, and reaction times.
5. **Adequate washing of wells is extremely important.** Inadequately washed wells will exhibit high background values, and may show false positive values. For manual washing, aspirate the contents of the wells, then fill each well with wash buffer solution. Avoid cross-contamination of the wells, particularly in the first wash after aspiration. Drain all of the wash buffer from the wells by inverting, then shaking residual wash buffer from the wells with a sharp “snapping” motion of the wrist. Repeat these steps for a total of 3 to 5 washes. The wells should then be rapped vigorously on a paper towel or other absorbent material to remove all traces of residual wash buffer. The use of an automated microwell washing system will assure consistent washing of the wells, and is recommended.
NOTE: Due to the various types of wash techniques and automated systems, the number of washes may be adjusted to obtain optimal results. Each laboratory should determine the most efficient number of washes for its washing system.
6. Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be rapped vigorously and blotted on absorbent paper or towels to minimize residual wash buffer.
7. Timing of all steps is critical. All serum samples should be diluted before beginning the procedure, and they must be dispensed into the microwells in as short a period of time as possible (not more than five minutes). Batch sizes should be set so that specimen handling can be accomplished comfortably within this time period.
8. With the exception of the last incubation (substrate solution), the start of each incubation period begins with completion of sample or reagent dispensing. The substrate solution incubation must be exactly 15 minutes for each well. All samples and reagents should be dispensed in the same sequence and at a constant rate.
9. Do not use Tween 20, Triton X-100, or other detergents in wash buffers or other reagents used in this assay.

INTERPRETATION OF RESULTS

CALCULATIONS

Subtract the absorbance value for the blank well from the absorbance values obtained in calibrator, control, and patient sample wells to obtain net absorbance values. Calculate the mean net absorbance values for duplicate wells.

QUALITY CONTROL

1. The mean net absorbance value of the calibrator must be at least 0.150. The mean net absorbance value of the calibrator is used as the cut-off value for the assay.
2. The blank control well should have an absorbance value of less than 0.100. Blank absorbance values greater than 0.100 indicate inadequate washing, or contamination of reagents.
3. The wells that contain positive control should give a net absorbance value greater than the cut-off value. The wells that contain negative control should give a net absorbance value less than the cut-off value. If the controls do not correspond to these limits, the test is invalid and must be repeated.
4. The cut-off value must be obtained for each run. Using a cut-off value from another run will invalidate the results.

INTERPRETATION OF PATIENT RESULTS

1. Patient sample wells that have net absorbance values greater than or equal to the cut-off value are considered to be positive for anticardiolipin antibodies. Patient sample wells that have net absorbance values less than the cut-off value are considered to be negative for anticardiolipin antibodies.

2. Sample wells that have net absorbance values below, but within 5% of the cut-off value are considered borderline. Borderline results should be repeated, or should be tested for specific IgG and IgM anticardiolipin antibodies using Immuno Concepts RELISA[®] Cardiolipin IgG & IgM Antibody Test System, catalog number 7096-02.

REPORTING OF RESULTS

Results should be reported as positive or negative for anticardiolipin antibodies.

EXPECTED VALUES

EXPECTED ANTIBODY PREVALENCE

Numerous studies have shown the association of anticardiolipin antibodies and Systemic Lupus Erythematosus (1, 7, 8, 11-16). In these studies, the prevalence of IgG anticardiolipin antibodies ranged from 23% to 54% (mean 41.4%), and the levels of IgM anticardiolipin antibodies ranged from 5% to 41% (mean 25.5%). The differences in prevalence seen among these studies are probably due to patient selection criteria and the patient populations studied. The Immuno Concepts RELISA[®] Cardiolipin Antibody Screening Test System was used to test serum samples from sixty patients who were seen for rheumatological consult. This patient population was selected because of clinical rheumatic diseases, but not for any specific disease state. In this population, twenty-three samples (38.3%) were positive for anticardiolipin antibodies.

LIMITATIONS OF THE TEST

1. Diagnosis cannot be made on the basis of anticardiolipin antibody titers alone. The physician must interpret these results in conjunction with the patient's history and symptoms, the physical findings, and other diagnostic procedures.
2. Treatment should not be initiated on the sole basis of a positive test for anticardiolipin antibodies. Clinical indications, other laboratory findings, and the physician's clinical impression must be considered before any treatment is initiated.
3. If the patient is negative for anticardiolipin antibodies, but the clinical findings suggest the presence of antiphospholipid antibodies, some investigators recommend testing for the lupus anticoagulant as a confirmation of the negative anticardiolipin results. If either the anticardiolipin antibody or the lupus anticoagulant results are positive, the patient is considered positive for antiphospholipid antibodies (1).
4. Patients with serologically positive syphilis infections may show a positive result for anticardiolipin antibodies. These patients are generally considered as not having increased thrombotic risk as is seen in rheumatic disease patients with anticardiolipin antibodies. Seventeen samples from patients with confirmed active or serofast syphilis (FTA-Abs and/or MHA-TP positive) were tested in the Immuno Concepts RELISA[®] Cardiolipin Antibody Screening Test System. Thirteen (76.5%) of these samples were positive for anticardiolipin antibodies. The diagnosis of syphilis should be confirmed or ruled out by specific antitreponemal antibody assays.
5. Anticardiolipin antibodies can occur transiently during many infections. If a patient tests positive while there are clinical signs of infection, the test should be repeated after the infection has been resolved.
6. This is a qualitative test that determines the presence or absence of anticardiolipin antibodies. This test cannot be used to determine quantitative levels of anticardiolipin antibodies.
7. This test is a screening test that uses a polyvalent anti-human immunoglobulin conjugate. The test will detect anticardiolipin antibodies of the IgG, IgM, or IgA classes, but will not differentiate between them.

PERFORMANCE CHARACTERISTICS

CLINICAL SPECIFICITY

Normal Controls: Serum specimens from 141 healthy blood donors were tested using the Immuno Concepts RELISA[®] Cardiolipin Antibody Screening Test System. Five of these samples were positive for anticardiolipin antibodies. Based on this unselected normal population, the Immuno Concepts RELISA[®] Cardiolipin Antibody Screening Test System is 96.5% specific for anticardiolipin antibodies.

Rheumatic Disease Controls: Serum specimens from twenty patients with rheumatic diseases other than SLE, and no history of thrombotic episodes, were tested in the Immuno Concepts RELISA[®] Cardiolipin Antibody Screening Test System. None of these samples were positive for anticardiolipin antibodies. The specificity of the assay in this selected population was 100%.

CLINICAL SENSITIVITY

The Immuno Concepts RELISA® Cardiolipin Antibody Screening Test System was used to test serum samples from sixty patients who were seen for rheumatological consult. This patient population was selected because of clinical rheumatic diseases, but not for any specific disease state. In this population, twenty-three samples (38.3%) were positive for anticardiolipin antibodies.

Serum specimens from twenty patients with SLE, and a history of at least one thrombotic episode, were tested in the Immuno Concepts RELISA® Cardiolipin Antibody Screening Test System. Fifteen (75%) of these samples were positive for anticardiolipin antibodies.

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In the event of damage to the protective packaging, please contact Immuno Concepts prior to use.



Manufacturer



Authorized Representative in the European Community



Temperature Limitation



Contains Sufficient for <n> tests



Consult Instructions for Use



In Vitro Diagnostic Medical Device



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Cat 7096-01-I,

4.11.02.003.098-En

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RELISA® CARDIOLIPIN SCREENING TEST

All samples, reagents (including the wash buffer solution), and microwells must be at room temperature before use.

1. PREPARE WORKSHEET

Label the worksheet that is enclosed in the kit to indicate the location of the samples in the microwells. Assay the calibrator and controls in duplicate. One well is used as a reagent. We recommend that each patient sample, calibrator, and control be assayed in duplicate until you have established an acceptable precision for the assay in your laboratory.

2. RECONSTITUTE WASH BUFFER (PBS)

Dissolve contents of one buffer pouch in one liter of deionized or distilled water. The PBS may be covered and stored at 2-25°C up to four weeks.

3. DILUTE PATIENT SAMPLES

Dilute patient samples 1:100 by adding 10 µl of serum to 990 µl of Sample Diluent. Mix well. The controls and calibrator are provided at the working dilution and do not require any further dilution.

4. PREPARE MICROWELLS

Remove the required number of microwell strips from the pouch and place them in the frame holder. The microwells must be firmly seated in the frame holder. Press down firmly on both ends of the strips so that they securely snap into the frame holder. If using individual wells or less than a full strip of wells, be sure each well is firmly seated. Wells that are properly seated in the frame holder will not fall out when the frame holder is inverted. If fewer than eight wells are needed for testing, the wells can be separated by snapping them apart. Unused wells can be returned to the foil pouch, sealed with the zipper seal, and refrigerated for up to 45 days.

5. DISPENSE SERUM DILUTIONS

Dispense 100 µl of the calibrators, controls, and patient samples into the appropriate wells as outlined on the worksheet. Dispense 100 µl of Sample Diluent into the reagent blank well.

6. INCUBATE MICROWELLS (30 minutes at room temperature, i.e., 18-25°C)

Incubate at room temperature for 30 minutes. The wells should be protected from drafts or shifts in temperature during incubation. If desired, the wells can be covered with transparent tape or a paper towel to protect them from dust or other foreign bodies.

7. WASH MICROWELLS (See General Procedural Notes 5 and 6)

Wash the wells 3 to 5 times with PBS Wash Buffer Solution. For manual washing, aspirate the contents of the wells, then fill each well with Wash Buffer Solution. Avoid cross-contamination of the wells, particularly in the first wash after aspiration. Drain all of the Wash Buffer from the wells by inverting, then shaking residual Wash Buffer from the wells with a sharp "snapping" motion of the wrist. Repeat these steps for a total of 3 to 5 washes. The wells should then be rapped vigorously on a paper towel or other absorbent material to remove all traces of residual Wash Buffer.

8. DISPENSE ENZYME ANTIBODY REAGENT

Dispense 100 µl of Enzyme Antibody Reagent to each of the wells.

9. INCUBATE MICROWELLS (30 minutes at room temperature, i.e., 18-25°C)

Incubate at room temperature for 30 minutes. The wells should be protected from drafts or shifts in temperature during incubation. If desired, the wells can be covered with transparent tape or a paper towel to protect them from dust or other foreign bodies.

10. WASH MICROWELLS

Wash the wells 3 to 5 times with PBS Wash Buffer Solution. For manual washing, aspirate the contents of the wells, then fill each well with Wash Buffer Solution. Avoid cross-contamination of the wells, particularly in the first wash after aspiration. Drain all of the Wash Buffer from the wells by inverting, then shaking residual Wash Buffer from the wells with a sharp "snapping" motion of the wrist. Repeat these steps for a total of 3 to 5 washes. The wells should then be rapped vigorously on a paper towel or other absorbent material to remove all traces of residual Wash Buffer.

11. DISPENSE SUBSTRATE SOLUTION

Using a timer to assure consistent intervals, dispense 100 µl of Substrate Solution to each of the wells. The Substrate Solution must be added to the wells at a steady rate, so that each well is incubated for exactly the same length of time (15 minutes). The Substrate Solution in wells incubated with positive samples will turn blue, and the solution in wells incubated with negative samples will be colorless to very pale blue.

12. INCUBATE MICROWELLS (Exactly 15 minutes at room temperature, i.e., 18-25°C)

Incubate at room temperature for exactly 15 minutes. The wells should be protected from drafts or shifts in temperature during incubation.

13. DISPENSE STOPPING REAGENT

After the first well has incubated for exactly 15 minutes, add 100 µl of Stopping Reagent to each well, in the same order and at the same rate that the Substrate Solution was added to the wells. Upon addition of Stopping Reagent, blue substrate solution will turn yellow and colorless solution will remain colorless.

14. READ ABSORBANCE OF WELLS

Within 30 minutes after addition of the Stopping Reagent, the wells must be read in a plate reading spectrophotometer. The wells are read at 450 nm against the reagent blank well. If a dual wavelength spectrophotometer is available, the wavelength for the reference filter should be 600-650 nm. Reading the microwells at 450 nm without a reference filter will result in higher absorbance values.

FOR TECHNICAL ASSISTANCE:

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