 **AccuCardia™ ELISA Test System**

**REF**

**CE Mark.bmp ACC6301**

**Rx Only**

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| Institute Name | Date |
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**PRINCIPLE OF THE ASSAY**

The AccuCardia test is a dual monoclonal antibody sandwich ELISA assay designed to quantitatively detect sPLA2-IIA protein in human plasma. A brief overview of the test procedure is as follows:

1. Test plasma, Calibrators, and Controls are diluted in Sample Diluent, then transferred to a microtiter plate containing immobilized anti-sPLA2-IIA antibody.
2. The diluted samples are incubated for one hour in the antibody-coated microwells. Sample-derived sPLA2-IIA is bound to the plate via interaction with the immobilized antibody. After incubation, the wells are washed to remove unbound plasma components.
3. A solution containing horseradish peroxidase (HRP)-conjugated anti-sPLA2-IIA antibody is then added to each well, and the plate is incubated again for one hour. After incubation, the plate is washed to remove unbound HRP conjugate.
4. The microwells containing immobilized sPLA2-IIA and HRP-conjugate are incubated for 10 minutes with peroxidase substrate solution, and hydrolysis of the substrate produces a color change.
5. After this incubation, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution is directly proportional to the amount of sPLA2-IIA protein in the original test sample.

**TEST SYSTEM COMPONENTS**

**Materials Provided:**

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label.

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| **PLATE** | | 1. | Plate: 96 wells configured in twelve, 1 x 8-well, strips coated with a sPLA2-IIA specific antibody. The strips are packaged in a strip holder and sealed in an envelope with desiccant. |
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| **CONJ** | | 2. | Conjugate: sPLA2-IIA specific HRP-conjugated antibody. One, 15mL, white-capped, amber bottle. Ready to use (pH 7.5 ± 0.1). |
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| **CONTROL** | **I** | 3. | Low Positive Control: One **green**-capped vial. Lyophilized. |
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| **CONTROL** | **II** | 4. | Medium Positive Control: One **red**-capped vial. Lyophilized. |
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| **CAL** | **A** | 5. | Calibrator A: One **white-**capped vial. Lyophilized. |
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| **CAL** | **B** | 6. | Calibrator B: One **yellow**-capped vial. Lyophilized. |
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| **CAL** | **C** | 7. | Calibrator C: One **orange**-capped vial. Lyophilized. |
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| **CAL** | **D** | 8. | Calibrator D: One **blue**-capped vial. Lyophilized. |
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| **CAL** | **E** | 9. | Calibrator E: One **clear**-capped vial. Lyophilized. |
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| **CAL** | **F** | 10. | Calibrator F: One **purple**-capped vial. Lyophilized. |
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| **DIL** | **SPE** | 11. | Sample Diluent: One, 30mL green-capped bottle. Ready to use (pH 7.5 ± 0.1). |
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| **SOLN** | **TMB** | 12. | TMB: One, 15mL, amber-capped, amber bottle containing 3, 3’, 5, 5’ - tetramethylbenzidine (TMB). Ready to use. |
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| **SOLN** | **STOP** | 13. | Stop Solution: One, 15mL, red-capped, bottle containing 1M H2SO4, 0.7M HCl. Ready to use. |
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| **WASHBUF** | **10X** | 14. | Wash Buffer Concentrate (10X): One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (clear solution). Preparation: Dilute 1 part concentrate + 9 parts deionized or distilled water. **NOTE: 1X solution will have a pH of 7.2 ± 0.2.** |

**NOTES:**

1. The Stop Solution and Wash Buffer are not test system/lot number dependent and may be used interchangeably with any of the ZEUS ELISA™ Test Systems.
2. Test System also contains:
   1. Component Label containing lot specific information for the materials packaged inside the Test System box.
   2. Package Insert providing instructions for use.

**PRECAUTIONS**

1. For *in vitro* diagnostic use.
2. Follow normal precautions handling laboratory reagents. In case of contact with eyes, rinse immediately with copious amounts of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HbsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products 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”: Current Edition; and OSHA’s Standard for Bloodborne Pathogens (19).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 – 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Stop Solution is TOXIC if inhaled, has contact with skin or is swallowed. It can cause burns. In case of accident or feeling ill, seek medical advice immediately.
8. The TMB Solution is HARMFUL. It is irritating to eyes, the respiratory system and skin.
9. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, the respiratory system and skin.
10. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
11. Dilution or adulteration of these reagents may generate erroneous results.
12. Do not use reagents from other sources or manufacturers.
13. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
14. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
15. Avoid microbial contamination of reagents. Incorrect results may occur.
16. Cross contamination of reagents and/or samples could cause erroneous results.
17. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
18. Avoid splashing or generation of aerosols.
19. Do not expose reagents to strong light during storage or incubation.
20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
21. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
22. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
23. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
24. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate’s enzymatic activity.
25. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Software capable of computing results using a 4-parameter logistic curve-fitting equation
3. Incubator capable of 37°C incubation.
4. Sample dilution plate
5. Pipettes capable of accurately delivering 10 – 200µL.
6. Multichannel pipette capable of accurately delivering 50 – 200µL.
7. Reagent reservoirs for multichannel pipettes.
8. Microwell washing system.
9. Distilled or deionized water.
10. One liter graduated cylinder.
11. Serological pipettes.
12. Disposable pipette tips.
13. Paper towels.
14. Laboratory timer to monitor incubation steps.
15. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

**STORAGE CONDITIONS**

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| storage2-8.bmp | Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening – strips are stable for 30 days, as long as the indicator strips on the desiccant pouch remains blue. |
| Reconstituted Calibrators/Controls are stable for up to 30 days. |
| Conjugate – DO NOT FREEZE. |
| Unopened Test System, Calibrators, Positive Controls, TMB, Sample Diluent |
| storage2-25.bmp | Stop Solution: 2 – 25°C  Wash Buffer (1X): 20 – 25°C for up to 7 days, 2 – 8°C for 30 days.  Wash Buffer (10X): 2 – 25°C |

**SPECIMEN COLLECTION**

1. ZEUS Scientific recommends that the user carry out specimen collection using standard of practice phlebotomy techniques and in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only **EDTA plasma** obtained by approved aseptic venipuncture procedures (21, 22). Avoid using hemolyzed, lipemic, or bacterially contaminated plasma.
4. While CLSI has published recommended guidelines for sample storage (20), internal real-time sample stability studies have demonstrated that endogenous sPLA2-IIA is stable for up to 14 days in EDTA plasma when stored at 2 - 8°C, and for up to 7 days when stored at room temperature. An internal freeze/thaw study has also demonstrated that endogenous sPLA2-IIA in EDTA plasma is stable for at least 5 freeze/thaw cycles.

**ASSAY PROCEDURE**

1. **Setup:**
2. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C) for approximately 30 minutes.
3. If using the kit for the first time, reconstitute the lyophilized Calibrators and Controls as follows:
   1. Add 250µL of distilled water to each vial reconstituting from lowest to highest concentration and using a fresh disposable pipette tip each time.
   2. Allow the freshly reconstituted vials to sit for 10 - 15 minutes at room temperature.
   3. Vortex before use making certain there is no undissolved lyophilized material on the underside of the cap.

**NOTE: Once reconstituted, the Calibrators and Controls are stable for 30 days.**

1. Prepare 1X Wash Buffer by adding the contents of the 10X Wash Buffer bottle to 900mL of deionized water. Mix by gentle inversion.
2. Determine the number of microwells needed. For each run, wells must be assigned for duplicate analysis of six Calibrators, Control I and Control II. Assign additional wells for duplicate analysis of patient samples. Check software and reader requirements for the correct Calibrator/Control configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

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| **EXAMPLE PLATE SET-UP** | | | | | | |
|  | 1 | 2 | 3 | 4 | 5 | 6 |
| A | Calibrator A | Calibrator A | Patient Specimen 1 | Patient Specimen 1 | Patient Specimen 9 | Patient Specimen 9 |
| B | Calibrator B | Calibrator B | Patient Specimen 2 | Patient Specimen 2 | Etc. |  |
| C | Calibrator C | Calibrator C | Patient Specimen 3 | Patient Specimen 3 |  |  |
| D | Calibrator D | Calibrator D | Patient Specimen 4 | Patient Specimen 4 |  |  |
| E | Calibrator E | Calibrator E | Patient Specimen 5 | Patient Specimen 5 |  |  |
| F | Calibrator F | Calibrator F | Patient Specimen 6 | Patient Specimen 6 |  |  |
| G | Control I | Control I | Patient Specimen 7 | Patient Specimen 7 |  |  |
| H | Control II | Control II | Patient Specimen 8 | Patient Specimen 8 |  |  |

1. **Procedure:**
2. Dilute each Calibrator, Control, and patient specimen 1:11 in Sample Diluent (i.e.: 25μL sample + 250μL Sample Diluent).
3. To individual wells, add 100μL of each diluted Calibrator, Control, and patient specimen in duplicate according to the ELISA plate setup map shown above. (Note: Ensure the samples are properly mixed, and use a different pipette tip for each sample)
4. Incubate the plate **uncovered** at 37 ± 1°C for 60 ± 5 minutes. **NOTE: Do NOT seal the plate during incubation.**
5. Wash the microwell strips five times. For automated washing, set the dispensing volume to 350µL/well. Set the wash cycle for five washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
6. Add 100µL of the Conjugate to each well at the same rate and order as the specimens.
7. Incubate the plate **uncovered** at 37 ± 1°C for 60 ± 5 minutes. **NOTE: Do NOT seal the plate during incubation.**
8. Wash the microwell strips according to Step d. above in this procedure.
9. Add 100µL of TMB to each well at the same rate and order as the specimens.
10. Incubate the plate **uncovered** at room temperature (20 - 25°C) for 10 minutes ± 1 minute.
11. Stop the reaction by adding 100µL of Stop Solution to each well at the same rate and order as the TMB. Positive samples will turn from blue to yellow.
12. Set the microwell plate reader to read at a wavelength of 450nm and measure the optical density (OD) of each well. Read the plate within 30 minutes of the addition of the Stop Solution. Be sure the bottom of the plate has been wiped clean and dry to prevent interference with the OD reading.

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| **ABBREVIATED TEST PROCEDURE** |
| 1. Reconstitute Calibrators and Controls . |
| 1. Dilute Calibrators, Controls and samples 1:11 in Sample Diluent 2. Add diluted Calibrators, Controls, and samples to duplicate microwells - 100µL/well. 3. *Incubate uncovered 60 ± 5 minutes at 37° ± 1°C.* 4. Wash five times, invert on paper towel to remove residual liquid. 5. Add Conjugate - 100µL/well. |
| 1. *Incubate uncovered 60 ± 5 minutes at 37° ± 1°C.* |
| 1. Wash five times, invert on paper towel to remove residual liquid. |
| 1. Add TMB - 100µL/well. |
| 1. *Incubate uncovered 10 ± 1 minute at 20 - 25°C.* |
| 1. Add Stop Solution - 100µL/well. |
| 1. Read optical density at 450 nm within 30 minutes. |

**QUALITY CONTROL**

1. Each time the assay is performed, the Calibrators, Controls, and samples must be run in duplicate.
2. Additional control material may be tested per guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
3. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.
4. The Low and Moderate Positive Controls are intended to monitor for substantial reagent failure. The Controls should yield ng/mL values within the lot-specific ranges listed on the component label provided with each kit.
5. If either, or both, of the Controls fall outside the ranges listed on the lot-specific label, assay results may be invalid, and testing **must** be repeated.

**INTERPRETATION OF RESULTS**

1. Enter the lot-specific ng/mL value for each Calibrator into the appropriate section of the desired 4-parameter logistic curve fitting software. The ng/mL values for each Calibrator are listed on the component label provided with each kit. **NOTE: Be sure to program the software to calculate the mean ng/mL value using the OD values derived from duplicate analysis of each Calibrator, Control, and patient sample.**
2. Test results should be interpreted in conjunction with the patient’s clinical evaluation and, potentially, the results of other diagnostic procedures.
3. Cardiac risk analysis calculations have been performed on clinical samples using a 25ng/mL sPLA2-IIA EDTA plasma concentration as the cutpoint for increased risk based on information gathered during a pilot study previously performed by ZEUS.
4. **Clincal Results Reporting**: For measured results: a.) 0 up to 20ng/mL, report as <20ng/mL; b.) 20 - 200ng/mL, report as measured value; c.) >200ng/mL should be reported as >200 ng/mL. **In addition to the numerical result, an interpretive comment must also be reported.** For sPLA2-IIA EDTA plasma results <25ng/mL, report with the interpretive comment “Results of <25ng/mL are statistically normal and therefore such individuals are not representative of being at risk of a future MI”. Results ≥25ng/mL should be reported with the interpretive comment “Risk of myocardial infarction within one year may be 2 - 3 times higher than it would be if sPLA2-IIA was <25ng/mL”.
5. If desired, specimens with results exceeding 200ng/mL may be repeat-tested after serial dilution in Sample Diluent. Once the diluted sample yields two consecutive values within the quantitative range of the assay, the dilution factor can be used to back-calculate the actual value as follows: **Example:** 4p software assigns a “>525 ng/mL” result to a patient sample. To begin retesting, the plasma sample is serially diluted (1:2, 1:4, 1:8, 1:16, etc…) in Sample Diluent, and each dilution is tested in duplicate according to the standard procedure. The results after serial dilution and retesting are as follows: 1:2 = “>525ng/mL”, 1:4 = 350ng/mL, 1:8 = 175ng/mL. 1:16 = 87.5ng/mL. **Calculations:** Measured value x Dilution factor = Actual ng/mL, so 175 x 8 = 1400ng/mL or 87.5 x 16 = 1400ng/mL.

Calibrators (ng/mL)

**LIMITATIONS OF THE ASSAY**

1. Only **EDTA plasma** samples should be tested with this assay. Lithium heparin plasma has been demonstrated to be **incompatible** with the assay. Serum, and plasma derived from blood collection tubes containing other anticoagulants, have not been fully tested/validated, and thus should be avoided.
2. Whole blood is not an appropriate sample type for this assay.

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