KAMIYA BIOMEDICAL COMPANY

Pig Apolipoprotein A1 (APO-A1) ELISA

For the quantitative determination of pig APO-A1 in serum, plasma, cell culture fluid and other biological fluids

Cat. No. KT-90598

For Research Use Only. Not for use in diagnostic procedures.

Product Information

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INTENDED USE

This APO-A1 ELISA kit is intended for laboratory research use only and not for use in diagnostic or therapeutic procedures. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of APO-A1 in the sample, this APO-A1 ELISA kit includes a set of calibrators. The calibrators are assayed at the same time as the samples and allow the operator to produce a calibration curve of optical density versus APO-A1 concentration. The concentration of APO-A1 in the samples is then determined by comparing the O.D. of the samples to the calibration curve.

PRINCIPLE

The coated well immunoenzymatic assay for the quantitative measurement of APO-A1 utilizes a monoclonal anti-APO-A1 antibody and a APO-A1-HRP conjugate. The assay sample and buffer are incubated together with APO-A1-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color is inversely proportional to the APO-A1 concentration since APO-A1 from samples and APO-A1-HRP conjugate compete for the anti-APO-A1 antibody binding site. Since the number of sites is limited, as more sites are occupied by APO-A1 from the sample, fewer sites are left to bind APO-A1-HRP conjugate. Calibrators of known APO-A1 concentrations are run concurrently with the samples being assayed and a calibration curve is plotted relating the intensity of the color (O.D.) to the concentration of APO-A1. The APO-A1 concentration is interpolated from this calibration curve.

COMPONENTS

Reagents	Quantity
Microtiter Plate	96 wells
Calibrator 1 (0 ng/mL)	1
Calibrator 2 (100 ng/mL)	1
Calibrator 3 (250 ng/mL)	1
Calibrator 4 (500 ng/mL)	1
Calibrator 5 (1,000 ng/mL)	1
Calibrator 6 (2,500 ng/mL)	1
Enzyme Conjugate	1 x 6 mL
Substrate A	1 x 6 mL
Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (100X concentrate)	1 x 10 mL
Balance Solution	1 x 3 mL

Note: The balance solution is used only when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then the balance solution is a superfluous reagent.

STORAGE

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature before a centrifugation for 15 minutes at approximately 1,000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20℃ or -80℃.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g at 4℃ within 30 minutes of collection. Store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

Tissue homogenates

The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02 mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1,500 x g (or 5,000 rpm). Remove the supernate and assay immediately or aliquot and store samples at -20℃ or -80℃.

Cell lysates

Cells should be lysed according to the following directions.

1. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
2. Wash cells three times in PBS.
3. Cells were resuspended in PBS and subjected to ultrasonication 3 times. Alternatively, freeze cells at -20℃. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.
4. Centrifuge at 1,000 x g (or 3,000 rpm) for 15 minutes at 4℃ to remove cellular debris.
5. Assay immediately or store samples at -20℃ or -80℃.

Cell culture fluid and other biological fluids

Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

NOTE: Serum, plasma, and cell culture fluid samples to be used within 24 hours may be stored at 4°C, otherwise samples must be stored at -20°C (≤3 months) or -80°C (≤6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES.**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. 100 mL and 1 liter graduated cylinders.
4. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
5. 37℃ incubator.
6. Absorbent paper.
7. Distilled or de-ionized water
8. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log or semi-log, or log-logit as desired.

9. Tubes to prepare calibrator or sample dilutions.

Precautions

1. **Kamiya Biomedical Company** is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the calibration curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g. antibody targets conformational isotope rather than linear isotope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Fresh samples without long term storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Bring all kit components and samples to room temperature (18-25°C) before use.

Dispense 10 µL of balance solution into 100 µL specimens, mix and stand for one hour (The proportion of balance solution and specimens shall be no less than 1:10). (**NOTE:** This step is required when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then this step should be skipped.)

Wash Solution

Dilute 10 mL of Wash Solution concentrate (100X) with 990 mL of de-ionized or distilled water to prepare 1,000 mL of Wash Solution (1X).

ASSAY PROCEDURE

It is recommended that all Calibrators and Samples be added in duplicate to the Microtiter Plate.

1. Secure the desired number of coated wells in the holder then add 100 µL of Calibrators or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. Add 100 µL of PBS (pH 7.0-7.2) in the blank control well.
2. Add 50 µL of Conjugate to each well (NOT blank control well). Mix well. Mixing well in this step is important. Cover and incubate for 1 hour at 37°C.
3. Wash the Microtiter Plate using one of the specified methods indicated below:
4. Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of five washes. After washing, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.
5. Automated Washing: Wash plate five times with diluted wash solution (350-400 µL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
6. Add 50 µL Substrate A and 50 µL Substrate B to each well, subsequently. Cover and incubate for 15 minutes at 37°C. (Avoid sunlight).
7. Add 50 µL of stop solution to each well. Mix well.
8. Read the optical density (O.D.) at 450 nm using a microtiter plate reader immediately.

CALCULATION OF RESULTS

1. This calibration curve is used to determine the amount of an unknown sample. Construct a calibration curve by plotting the average O.D. (450 nm) for each calibrator on the horizontal (X) axis against the concentration on the vertical (Y) axis, and draw a best fit curve through the points on the graph.
2. First, calculate the mean O.D. value for each calibrator and sample. All O.D. values are subtracted by the mean value of the blank control before result interpretation. DO NOT subtract the O.D. of calibrator zero.
3. Use graph paper or statistical software to generate a four parameter logistic (4-PL) curve-fit or logit-log linear regression curve. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own calibration curve.
5. The sensitivity in this assay is 1.0 ng/mL.
6. This assay has high sensitivity and excellent specificity for detection of APO-A1. No significant cross-reactivity or interference between APO-A1 and analogues was observed.
   Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between APO-A1 and all the analogues, therefore, cross reaction may still exist in some cases.

Disposal Note Safety

1. Avoid ingestion and contact with eyes, skin, and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.
2. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

IMPORTANT NOTES

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 4°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all calibrators, controls, and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation time for all wells.
8. Cover or cap all reagents when not in use.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
13. Because stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between stabilized Chromogen and metal, otherwise color may develop.
14. Incomplete washing will adversely affect the test outcome. All washing must be performed with wash buffer provided.
15. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well.
16. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

17. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells.
18. If using an automated washer, the operating instructions for washing equipment should be carefully followed.
19. Assay Procedure Preliminary notes: Do not mix reagents from different lots. It is recommended that assays be performed in duplicate. Calibrators and samples must be assayed at the same time. Avoid exposing the substrate to direct sunlight.

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