KAMIYA BIOMEDICAL COMPANY

Rat apolipoprotein E (Apo-E) ELISA

For the quantitative determination of rat Apo-E in serum, plasma, tissue homogenates and cell culture supernates

Cat. No. KU-199

For Research Use Only.

Product Information
Rat apolipoprotein E (Apo-E) ELISA

Cat. No. KU-199

INTENDED USE

The Rat apolipoprotein E (Apo-E) ELISA is for the quantitative determination of rat Apo-E in serum, plasma, tissue homogenates and cell culture supernates. For research use only.

PRINCIPLE

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Apo-E. Calibrators or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated antibody preparation specific for Apo-E. The competitive inhibition reaction is launched between with pre-coated Apo-E and Apo-E in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of Apo-E in the samples. The color development is stopped and the intensity of the color is measured.

PERFORMANCE

Detection Range

15.6 ng/mL - 1,000 ng/mL.

Sensitivity

The minimum detectable dose of rat Apo-E is typically less than 15.6 ng/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D. value of 20 replicates of the zero calibrator added by their three standard deviations.

Specificity

This assay has high sensitivity and excellent specificity for detection of rat Apo-E. No significant cross-reactivity or interference between rat Apo-E and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between rat Apo-E and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-assay Precision (Precision within an assay): CV% <8%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV% <10%

Three samples of known concentration were tested in twenty assays to assess.

Limitations of the Procedure

- For research use only.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate higher values than the highest calibrator, dilute the samples and repeat the assay.
- Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

COMPONENTS

Reagents	Quantity
Assay plate	1 (96 wells)
Calibrator (Lyophilized)	2
HRP-conjugate (100 x concentrate)	1 x 60 µL
HRP-conjugate Diluent	1 x 10 mL
Sample Diluent	2 x 20 mL
Wash Buffer (25 x concentrate)	1 x 20 mL
TMB Substrate	1 x 10 mL
Stop Solution	1 x 10 mL
Plate sealer for 96 wells	4

STORAGE

Unopened kit: Store at 4℃. Do not use the kit beyond the expiration date.

Opened kit: May be stored up to 1 month at 4℃.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm - 570 nm.
2. An incubator which can provide stable incubation conditions up to 37℃ ± 0.5℃.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100 mL and 500 mL graduated cylinders.
6. De-ionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.

Precautions

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4℃ before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

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. Assay immediately or aliquot and store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

Tissue Homogenates

100 mg tissue was rinsed with 1X PBS, homogenized in 1 mL of 1X PBS and stored overnight at -20℃. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5,000 x g, 4℃. The supernate was removed and assayed immediately. Alternatively, aliquot and store samples at -20℃ or -80℃. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Cell Culture Supernates

Remove particulates by centrifugation for 15 minutes at 1000 x g, 4℃ and assay immediately or aliquot and store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

- Recommend to dilute the serum or plasma samples with Sample Diluent (1:200) before test. The suggested 200-fold dilution can be achieved by adding 5 µL sample to 45 µL of Sample Diluent. Complete the 200-fold dilution by adding 15 µL of this solution to 285 µL of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.
- For tissue homogenates and cell culture supernates samples, recommend to determine the dilution factor by pretest. The optimal dilution factor should be determined by users according to their particular experiments.

Note:

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. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
5. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
6. 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.
7. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
8. 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.
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

REAGENT PREPARATION

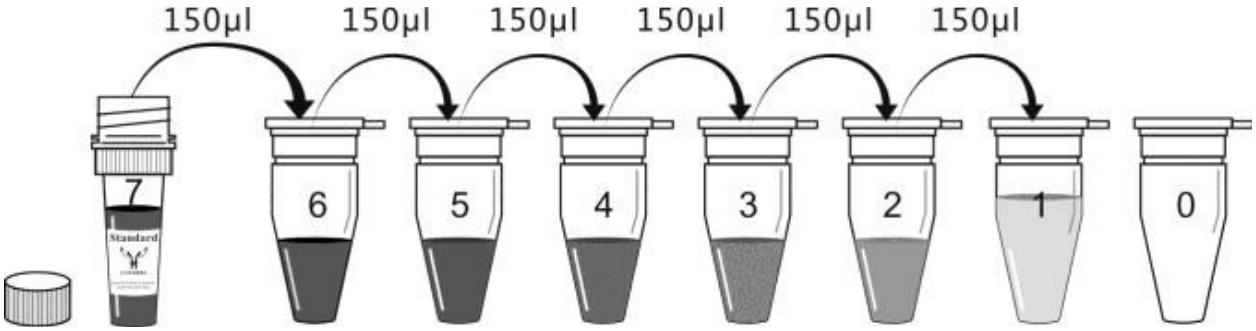
Note:

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**
- Bring all reagents to room temperature (18-25℃) before use for 30 min.
- Prepare fresh calibrator for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Calibrators according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

1. **HRP-conjugate (1x)-** Centrifuge the vial before opening.
HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of **HRP-conjugate** + 990 µL of **HRP-conjugate Diluent**.

2. **Wash Buffer (1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).

3. **Calibrator**
Centrifuge the calibrator vial at 6,000-10,000 rpm for 30 s.
Reconstitute the **Calibrator** with 1.0 mL of **Sample Diluent**. Do not substitute other diluents. This reconstitution produces a stock solution of 1,000 ng/mL. Mix the calibrator to ensure complete reconstitution and allow the calibrator to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.
Pipette 150 µL of **Sample Diluent** into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Calibrator serves as the high calibrator (1,000 ng/mL). **Sample Diluent** serves as the zero calibrator (0 ng/mL).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ml	1000	500	250	125	62.5	31.2	15.6	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and calibrators be assayed in duplicate.

1. Prepare all reagents, working calibrators, and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4℃.
3. Set a Blank well without any solution.
4. Add 50 µL of calibrator and sample per well. Then add 50 µL of **HRP-conjugate (1x)** to each well (Not to Blank well). Cover the microtiter plate with adhesive strip. Incubate for 30 minutes at 37℃.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 90 µL of **TMB Substrate** to each well. Incubate for 20 minutes at 37℃. **Protect from light.**
7. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*__Samples may require dilution. Please refer to Sample Preparation section.__

Note:

1. The final experimental results will be closely related to operation skills of the end users and the experimental environments.
2. Sample or reagents addition: Please use the freshly prepared Calibrator. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without

interruption. Duplication of all calibrators and samples, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each calibrator level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes). TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples and calibrators as instructed.
2. Set a Blank well without any solution.
3. Add 50 µL calibrator or sample to each well.
4. Add 50 µL HRP-conjugate (1x) to each well (not to Blank well).
5. Incubate for 30 minutes at 37℃.
6. Aspirate and wash 5 times.
7. Add 90 µL TMB Substrate to each well. Incubate 20 minutes at 37℃. **Protect from light.**
8. Add 50 µL Stop Solution to each well. Read at 450 nm within 5 minutes.

CALCULATION OF RESULTS

Using professional graphing software to make a calibration curve is recommended.

Average the duplicate readings for each calibrator and sample and subtract the average optical density of Blank.

Create a calibration curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a calibration curve by plotting the mean absorbance for each calibrator on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Apo-E concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the calibration curve must be multiplied by the dilution factor.

FOR RESEARCH USE ONLY.

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com