# HUMAN APOLIPOPROTEIN A-IV (APOA-IV) ELISA KIT

**FOR THE QUANTITATIVE DETERMINATION OF HUMAN APOA-IV CONCENTRATIONS IN EDTA PLASMA AND SERUM**

**THIS PROTOCOL OR DATA IS PROVIDED FOR DEMONSTRATION ONLY.**
**ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.**

**FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

**PURCHASE INFORMATION:**
**THIS KIT IS FOR ONE TIME USE ONLY.**

| ELISA NAME | HUMAN APOA-IV ELISA |
|---|---|
| Catalog No. | SK00401-01 |
| Lot No. | |
| Formulation | 96 T |
| Standard range | 15.6 - 2000 ng/mL |
| Sensitivity | 2 ng/mL |
| Sample Volume | 100 µL |
| Dilution Factor | Optimal dilutions should be determined by each laboratory for each application |
| Sample Type | Serum, EDTA plasma |
| Specificity | Human APOA-IV only |
| Calibration | Human APOA-IV recombinant |
| Intra-assay Precision | 4 - 6% |
| Inter-assay Precision | 8 - 12% |
| Storage | 2 - 8° C for 1 month. More information check page 3 |
| This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol. | |

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## DESCRIPTION

This Human Apolipoprotein A-IV (APOA-IV) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human APOA-IV from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human APOA-IV and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural APOA-IV samples.

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human APOA-IV. The capture antibody can bind to the human APOA-IV in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human APOA-IV is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human APOA-IV bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

## PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

## COMPONENTS PROVIDED

| DESCRIPTION | CODE | QUANTITY |
|---|---|---|
| **APOA-IV Microplate** - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human APOA-IV. | **401-01-01** | **1 plate** |
| **APOA-IV Standard** – refer to lot of recombinant human APOA-IV in a buffered protein base with preservative; lyophilized. | **401-01-02** | **1 vial** |
| **Detection Antibody Concentrate** – refer to lot of 10-fold concentrate of biotinylated purified antibody against human APOA-IV with preservative; lyophilized. | **401-01-03** | **1 vial** |
| **Positive Control** – one vial of recombinant human APOA-IV; lyophilized. | **401-01-04** | **1 vial** |
| **Streptavidin-HRP Conjugate** – 120 µL/vial of 100-fold concentrated solution of Streptavidin conjugate to HRP. | **SAHRP** | **1 vial** |
| **Dilution Buffer** – 40 mL of buffered protein based solution with preservative. | **DB01** | **1 bottle** |
| **Antibody Diluent Solution** – 12 mL of buffered protein based solution with preservative. | **DB83** | **1 bottle** |
| **HRP Diluent Solution** – 12 mL of buffered protein based solution with preservative. | **DB08A** | **1 bottle** |
| **Wash Buffer** – 50 mL of 10-fold concentrated buffered surfactant, with preservative. | **WB01** | **1 bottle** |
| **TMB Substrate Solution** -11 mL of TMB substrate solution. | **TMB01** | **1 bottle** |
| **Stop Solution** – 11 mL of 0.5M HCl. | **S-STOP** | **1 bottle** |
| **Plate Sealer** | **EAPS** | **1 piece** |
| **Plastic Pouch** | **P01** | **1 piece** |

## STORAGE

**Unopened Kit:** Store at 2 – 8° C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer, Antibody Diluent Solution and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 – 8 °C. Do not use kit past expiration date.

## ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

## SAMPLE COLLECTION AND STORAGE

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C or -70° C. Avoid repeated freeze-thaw cycles.
**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C or -70° C. Avoid repeated freeze-thaw cycles.
**Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

## SAMPLE PREPARATION

**Optimal dilutions should be determined by each laboratory for each application.**
**Use polypropylene test tubes.**

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50mL of Wash Buffer Concentrate into deionized or distilled water (450mL) to prepare 500 mL of 1x Wash Buffer.

**APOA-IV Standard -** Reconstitute the APOA-IV standard with refer to lot of Dilution Buffer. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

| Tube | Standard | Dilution Buffer | Concentration |
|---|---|---|---|
| stock | powder | Refer to lot | 2000 ng/ml |
| # 1 | 250µl of stock | 250µl | 1000 ng/ml |
| # 2 | 250µl of 1 | 250µl | 500 ng/ml |
| # 3 | 250µl of 2 | 250µl | 250 ng/ml |
| # 4 | 250µl of 3 | 250µl | 125 ng/ml |
| # 5 | 250µl of 4 | 250µl | 62.5 ng/ml |
| # 6 | 250µl of 5 | 250µl | 31.25 ng/ml |
| # 7 | 250µl of 6 | 250µl | 15.6 ng/ml |

**Positive Control** - Reconstitute Positive Control with refer to lot of Dilution Buffer to prepare working solution.

**Detection Antibody -** Reconstitute the Detection Antibody Concentrate with refer to lot of **Antibody Diluent Solution (DB83)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB83)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

**Streptavidin-HRP Conjugate** - Pipette 11.88 mL of **HRP Diluent Solution (DB08A)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution ***(protect from light)***.

## ELISA PROTOCOL

**Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples,** or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. 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.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

Calculation of samples with a concentration exceeding that of standard 2000 ng/mL may result in inaccurate, low human soluble APOA-IV levels. Such samples require further external predilution according to expected human APOA-IV values with Dilution Buffer in order to precisely quantify the actual human APOA-IV level.

## TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

| Standard (ng/mL) | Average OD450nm (Corrected) |
|---|---|
| Blank | 0 (refer to lot) |
| 15.6 | 0.046 |
| 31.25 | 0.078 |
| 62.5 | 0.126 |
| 125 | 0.221 |
| 250 | 0.376 |
| 500 | 0.704 |
| 1000 | 1.250 |
| 2000 | 2.109 |

## SPECIFICITY

| PROTEINS | CROSS-REACTIVITY |
|---|---|
| Human APOA-IV | 100% |
| Human APOH | 0 |

## SUMMARY OF ASSAY PROCEDURE

| PREPARE REAGENTS, SAMPLES AND STANDARDS |
|---|
| ↓ |
| Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT. |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT. |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 1 hour on plate shaker at RT. **Protect from light**. |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µl Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light**. |
| ↓ |
| Add 100 µl Stop Solution to each well. Read at 450nm. |