HUMAN APOLIPOPROTEIN H (APOH) / BETA-2 GLYCOPROTEIN 1 (B2G1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN APOH CONCENTRATIONS IN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:
THIS IS FOR ONE TIME USE ONLY

ELISA NAME	HUMAN APOLIPOPROTEIN H (APOH) ELISA KIT
Catalog No.	SK00548-01
Lot No.	
Formulation	96 T
Standard Range	25 - 1600 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL of diluted samples
Sample Type	Serum, EDTA Plasma
Dilution Factor	400K ~ 800K **(Optimal dilutions should be determined by each laboratory for each application)**
Specificity	Human APOH
Calibration	Human APOH recombinant (HEK293 derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 10%
Storage	2 - 8° C for 2 months. See page 2-3 for detail
This kit contains sufficient materials to run 35 - 40 samples duplicated provided that assay is run according to protocol.	

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.

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DESCRIPTION

This Human Apolipoprotein H (APOH) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human APOH from serum and plasma in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human APOH. The capture antibody can bind to the human APOH in the standard and samples. After washing the plate of any unbound substances, an antibody-HRP conjugate against human APOH is added to the wells. 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 APOH 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 with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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
APOH Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human APOH.	**548-01-01**	**1 plate**
APOH Standard – 1200 pg/vial of recombinant human APOH in a buffered protein base with preservative; lyophilized.	**548-01-02**	**1 vial**
Detection Antibody-HRP Conjugate – 140 µL/vial of 75-fold concentrated solution of antibody conjugated to HRP against human APOH.	**548-01-03**	**1 vial**
Positive Control – one vial of recombinant human APOH; lyophilized.	**548-01-04**	**1 vial**
Dilution Buffer – 25 mL of 10-fold concentration buffered protein based solution with preservative.	**DB02T**	**1 bottle**
Antibody HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08B**	**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.	**TMB03**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 2 months. For longer storage for up to 6 months, unopened Standard, Positive Control, Dilution Buffer concentrate and Antibody HRP Diluent Solution should be stored at -20 °C.
Detection Antibody-HRP Conjugate 75-fold concentrated solution and TMB Substrate Solution should be stored only at -20° C or -70° C. (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other

components can be stored at 2 – 8° C for up to 6 months. Do not use kit past expiration date.
Microplate Wells: Return unused microplate strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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. 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. 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

Serum and plasma samples may require at least a 400000 (400K) ~ 800000 (800K)-fold dilution. A suggested 100-fold dilution is 10 µL sample + 990 µL 1x Dilution Buffer. A suggest 10,000-fold dilution is 10 µL of 100-fold diluted sample + 990 µL 1x Dilution Buffer. A suggested 400,000-fold dilution is 10 µL of 10,000-fold diluted sample + 390 µL 1x Dilution Buffer. A suggested 800,000-fold dilution is 125 µL of 400,000-fold diluted sample + 125 µL 1x Dilution Buffer. Please refer more information on page 5.

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.

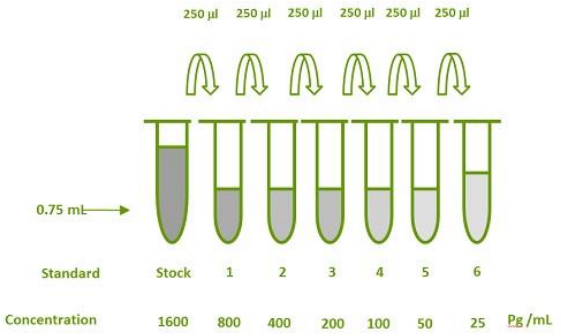
1x Dilution Buffer – Warm to room temperature or warm it in 37°C water bath for 20 min. Dilute 25 mL of 10-fold concentrated **Dilution Buffer Concentrate** (**DB02T**) into deionized or distilled water (225 mL) to prepare 250 mL of 1x Dilution Buffer (DB02).

Wash Buffer - If crystals have formed in the concentrate, Warm to room temperature or warm it in 37°C water bath for 20 min. and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

APOH Standard - Reconstitute the APOH Standard with **0.75 mL** of 1x Dilution Buffer. This reconstitution produces a stock solution of 1600 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **1600 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	750 µl	1600 pg/ml
# 1	250 µl of stock	250 µl	800 pg/ml
# 2	250 µl of 1	250 µl	400 pg/ml
# 3	250 µl of 2	250 µl	200 pg/ml
# 4	250 µl of 3	250 µl	100 pg/ml
# 5	250 µl of 4	250 µl	50 pg/ml
# 6	250 µl of 5	250 µl	25 pg/ml

Positive Control - Reconstitute the Positive Control with 1.0 mL of 1x Dilution Buffer. **NOTE:** Positive control could be reused within a few days if stored at -20° C ~ -70° C.



Detection Antibody-HRP Conjugate - Pipette **10.360** mL of **Antibody HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 140 µL of 75-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Detection Antibody-HRP conjugate should be used within 1-2 hours **(protect from light)**. **DO NOT FREEZE.**

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. 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 (400 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.
6. Add 100 µL of Detection Antibody-HRP conjugate working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. **Protect from light.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 20-25 minutes on microplate shaker at room temperature. **Protect from light.**
9. 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.
10. Determine the optical density of each well within 2 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

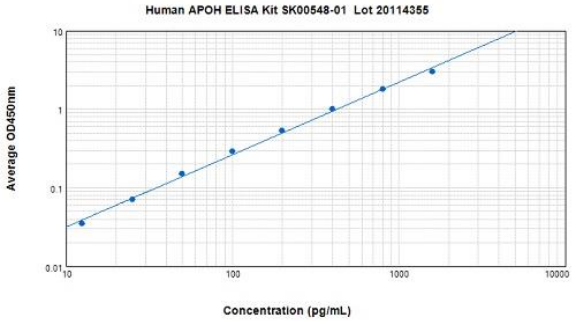
Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately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

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

TYPICAL DATA

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

Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.050)
12.5 (optional)	0.035
25	0.070
50	0.151
100	0.291
200	0.539
400	0.993
800	1.819
1600	3.014



SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Apolipoprotein H (HEk293)	100
Human Apolipoprotein J/Clusterin (HEK293)	0
Human APOA-4	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well Detection Antibody-HRP working solution to each well. Incubate 2 hours on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 20-25 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 2 min.

Use 10 µL of Human serum or plasma samples to prepare 1:200K, 1: 400K or 800K dilution.

		Final Dilution
10 µL of Human sample	990 µL of 1x Dilution Buffer (DB02)	**100**
10 µL of 100-fold diluted sample solution	990 µL of 1x Dilution Buffer (DB02)	**10000 (10K)**
15 µL of 10000-fold diluted sample solution	285 µL of 1x Dilution Buffer (DB02)	**200000(200K)**
10 µL of 10000-fold diluted sample solution	390 µL of 1x Dilution Buffer (DB02)	**400000(400K)**
125 µL of 400K-fold diluted sample solution	125 µL of 1x Dilution Buffer (DB02)	**800000(800K)**

Use 5 µL of Human serum or plasma samples to prepare 1:200K, 1: 400K or 800K dilution.

		Final Dilution
5 µL of Human sample	995 µL of 1x Dilution Buffer (DB02)	**200**
10 µL of 200-fold diluted sample solution	990 µL of 1x Dilution Buffer (DB02)	**20000 (20K)**
30 µL of 10000-fold diluted sample solution	270 µL of 1x Dilution Buffer (DB02)	**200000(200K)**
15 µL of 10000-fold diluted sample solution	285 µL of 1x Dilution Buffer (DB02)	**400000(400K)**
125 µL of 400K-fold diluted sample solution	125 µL of 1x Dilution Buffer (DB02)	**800000(800K)**

The research samples were diluted by 1 x Dilution Buffer (DB02). Its linearity and recovery was assayed by Human APOH ELISA Kit SK00548-01.

Sample	Dilution factor	Assayed (pg/mL)	Final (µg/mL)	Recovery %
Serum	200K	878.004	175.600	100
Serum	400K	456.608	182.643	104
Serum	800K	214.468	171.574	98
EDTA Plasma	200K	1208.363	241.672	100
EDTA Plasma	400K	598.017	239.206	99
EDTA Plasma	800K	319.257	255.405	106

Well Position: