HUMAN ADIPONECTIN (TOTAL) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ADIPONECTIN CONCENTRATIONS IN CELL CULTURE SUPERNATES, PLASMA AND SERUM

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.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN ADIPONECTIN ELISA**
Catalog No.	SK00010-02
Lot No.	
Formulation	96 T
Standard Range	1.56-100 ng/mL
Sensitivity	200 pg/mL
Sample Volume	100 µL of diluted samples
Sample Dilution	**1000 (Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, Plasma, Cell Culture Supernates
Specificity	Human Adiponectin only
Calibration	Human Adiponectin Fc Recombinant (HEK293)
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Adiponectin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Adiponectin from cell culture supernates, serum and plasma in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Adiponectin. The capture antibody can bind to the human Adiponectin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Adiponectin 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 Adiponectin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURE 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.
_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
Adiponectin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human Adiponectin.	**010-02-01**	**1 plate**
Adiponectin Standard – 100 ng/vial of recombinant human Adiponectin in a buffered protein base with preservative; lyophilized.	**010-02-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated purified antibody against human Adiponectin with preservative; lyophilized.	**010-02-03**	**1 vial**
Positive Control – one vial of recombinant human Adiponectin; lyophilized.	**010-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB01**	**2 bottles**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08C**	**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 10 months. For longer storage, unopened Standard, Positive

Control and Detection Antibody Concentrate, Dilution Buffers (DB01) and HRP Diluent Solution (DB68C) should be stored at -20° C or -70° 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.

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

Cell Culture Supernates – Centrifuge and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order Code: 00700-01-25, 25 TIU) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may need a 1000 fold dilution. A suggested 50-fold dilution is 10 µL sample + 490 µL Dilution Buffer. Then, to make a final 1000-fold dilution is 12.5 µL of 50-fold diluted sample + 237.5 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application with a pretest. 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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Adiponectin Standard - Reconstitute the Adiponectin standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 100 ng/mL. 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 #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **100 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	1.0 ml	100 ng/ml
# 1	250µl of stock	250µl	50 ng/ml
# 2	250µl of 1	250µl	25 ng/ml
# 3	250µl of 2	250µl	12.5 ng/ml
# 4	250µl of 3	250µl	6.25 ng/ml
# 5	250µl of 4	250µl	3.125 ng/ml
# 6	250µl of 5	250µl	1.56 ng/ml

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control working solution.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer 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 10.89 mL of **HRP Diluent Solution (DB08C)** into a 15 mL

centrifuge tube and transfer 110 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 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 90 minutes 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 45 minutes 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 20-25 minutes 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 within 3 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-parameter curve fit.

If samples have been diluted by 1000, the concentration read from the standard curve must be multiplied by the dilution factor 1000.

TYPICAL STANDARD CURVE

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

ADIPONECTIN (NG/ML)	CORRECTED (450NM)
Blank	0 (0.101)
1.56	0.032
3.125	0.079
6.25	0.139
12.5	0.289
25	0.472
50	0.996
100	1.896

LINEARITY

To assess the linearity of the assay, pooled research human serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (UG/ML)	RECOVERY (%)
1000 X	10.129	10.129	100
2000 X	5.247	10.494	104

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (UG/ML)	RECOVERY (%)
1000 x	9.108	9.108	100
2000 x	4.620	9.240	101

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Adiponectin Fc Rec. (HEK Derived)	100%
Human Adiponectin Rec. (CHO derived)	0
Mouse Adiponectin Rec. (HEK293 derived)	0
Human CTRP6	0

The human adiponectin recombinant derived from E. Coli may not be detected by this ELISA Kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of Dilution Buffer to blank wells.
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate for 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Detection Antibody working solution to each well. Incubate for 90 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 45 min on 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 3 min.