HUMAN ADIPSIN ELISA KIT

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

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 ADIPSIN ELISA
Catalog No.	SK00024-01
Lot No.	
Formulation	96 T
Standard range	8 - 512 pg/mL
Sensitivity	1 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Dilution Factor	8000 or higher (**Optimal dilutions should be determined by each laboratory for each application**)
Specificity	Human Adipsin
Calibration	Human Adipsin (HEK293 cells derivd) recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 – 8°C

This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
            Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

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

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a mouse monoclonal antibody specific for human Adipsin. The capture antibody can bind to the human Adipsin in the standard and samples. After washing the plate of any unbound substances, a biotinylated polyclonal antibody against human Adipsin 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 Adipsin 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.
_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
Adipsin Microplate - 96 well microplate coated with a mouse monoclonal antibody against Adipsin.	**024-01-01**	**1 plate**
Adipsin Standard – 10 ng/vial of lyophilized recombinant human Adipsin.	**024-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of lyophilized biotinylated polyclonal antibody against Adipsin.	**024-01-03**	**1 vial**
Positive Control - one vial of lyophilized recombinant human Adipsin.	**024-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 50 mL of buffered protein based solution with preservative.	**DB01**	**2 bottles**
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 vial**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate 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

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) (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 at least a *8000-fold dilution*. A suggested **50-fold dilution** is 10 μL sample + 490 μL Dilution Buffer, then to make **1000-fold dilution** is 10 μL of 50-fold diluted sample + 180 μL Dilution Buffer. Lastly, to make **8000-fold dilution** is 70 μL of 1000-fold diluted sample + 210 μL Dilution Buffer.
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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Adipsin Standard - Reconstitute the Adipsin standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 10000 pg/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 **512 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	1000 μl	10000 pg/ml
# 1	25.6 μl of stock	474.4 μl	512 pg/ml
# 2	250 μl of 1	250 μl	256 pg/ml
# 3	250 μl of 2	250 μl	128 pg/ml
# 4	250 μl of 3	250 μl	64 pg/ml
# 5	250 μl of 4	250 μl	32 pg/ml
# 6	250 μl of 5	250 μl	16 pg/ml
# 7	250 μl of 6	250 μl	8 pg/ml

Positive Control – Reconstitute the Positive Control with 2.0 mL of Dilution Buffer.

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 11.88 mL of **HRP Diluent Solution (DB08A)** into a 15 mL centrifuge tube and transfer 120 μL of 100-fold

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concentrated stock solution to prepare working solution **(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. Add 100 µL of **Dilution Buffer** to Blank wells.
3. Add 100 µL of **Standard dilutions**, **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 60 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 20-24 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 15 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.

SPECIFICITY

Proteins	Cross-reactivity (%)
Human Adipsin (HEK293)	100
Human Adipsin (NS0)	100
Human Coagulation Factor II	0
Human Coagulation Factor X	0
Human Coagulation Factor XI	0

TYPICAL DATA

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

ADIPSIN (PG/ML)	ABSORBANCE 450NM (CORRECTED)
Blank	0 (0.138)
8	0.060
16	0.112
32	0.207
64	0.407
128	0.759
256	1.218
512	1.600

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 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 60 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 20-24 min on the plate shaker at RT. **Protect from light.**
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.