HUMAN OMENTIN 1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN OMENTIN 1 CONCENTRATIONS IN SERUM, PLASMA AND CELL CULTURE SUPERNATES OR TISSUES

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 OMENTIN 1 ELISA**
Catalog No.	SK00020-01
Lot No.	
Formulation	96 T
Standard range	0.5 - 32 ng/mL
Sensitivity	125 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma, Cell Culture Supernates or Tissues
Specificity	Human Omentin 1 only
Calibration	Human Omentin 1 recombinant
Pretreatment	Required
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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

DESCRIPTION

This Human Omentin 1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Omentin 1 from cell culture supernates or tissues, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Omentin 1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Omentin 1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Omentin 1. The capture antibody can bind to the human Omentin 1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Omentin 1 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 (TMB) is added to the wells and color develops in direct proportion to the amount of human Omentin 1 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.
_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
Omentin 1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against Omentin 1.	**020-01-01**	**1 plate**
Omentin 1 Standard – 32 ng/vial of recombinant human Omentin 1 in a buffered protein base with preservative; lyophilized.	**020-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against Omentin 1 with preservative; lyophilized.	**020-01-03**	**1 vial**
Positive Control – one vial of recombinant human Omentin 1; lyophilized.	**020-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugated to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
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**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage for up to 8 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent

Solution should be stored at -20° C or -70° C. **Streptavidin-HRP Conjugate** 100-fold concentrated 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.
- **500mM TCEP (fresh preparation) Soltec Ventures, Product #: M115**

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 - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000x 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 1000x 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

Samples must be pretreated prior to assay. Use Dilution Buffer with 10mM TCEP to dilute and treat samples. Standard and Positive Control ***DO NOT NEED*** *Pretreatment.*

1. Add 240 µl of 500 mM TCEP to 11.76 mL of **Dilution Buffer (DB06)** to prepare **Pretreatment Solution** (10mM TCEP in **Dilution Buffer DB06**). (12 mL for ~50 samples pretreatment).
1. Add 50 µL of sample to 200 µl of **Pretreatment Solution** in a polypropylene tube. **Note:** This pretreatment dilution (5-fold dilution) may require optimization.
2. Vortex gently and incubate for 30 minutes at room temperature. Assay immediately and discard any excess pretreated samples.

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

REAGENT PREPARATION

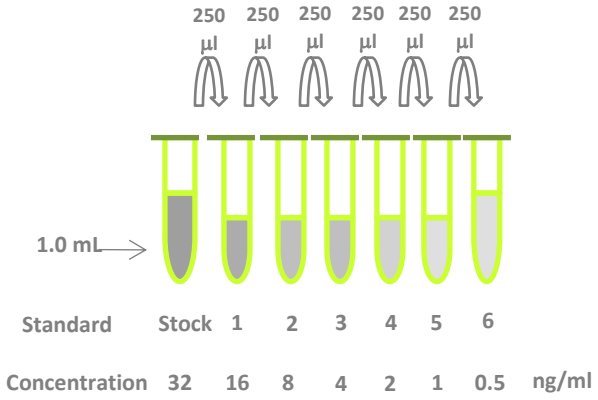
Bring all reagents to room temperature before use.

Dilution Buffer (DB06) - Dilution Buffer (DB06) is highly viscous, **warm in 30 - 37° C water bath** until liquid flows more freely**.**

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.

Omentin 1 Standard - Reconstitute the Omentin 1 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 32 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 (see below). Mix each tube thoroughly before the next transfer. The **32 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	1000 µl	32 ng/ml
# 1	250 µl of stock	250 µl	16 ng/ml
# 2	250 µl of 1	250 µl	8 ng/ml
# 3	250 µl of 2	250 µl	4 ng/ml
# 4	250 µl of 3	250 µl	2 ng/ml
# 5	250 µl of 4	250 µl	1 ng/ml
# 6	250 µl of 5	250 µl	0.5 ng/ml



Positive Control – Reconstitute the Positive Control with 1.0 mL Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 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 DB08C 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 5-10 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 using a microplate reader set to 450 nm within 3 minutes.

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. The data may be linearized by plotting the log of the Omentin 1 concentrations versus the log of the O.D. 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 the 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 (NG/ML)	AVERAGE OD450 NM (CORRECTED)*
Blank	0 (0.089)
0.5	0.046
1	0.099
2	0.171
4	0.324
8	0.616
16	1.109
32	1.912

SPECIFICITY

Protein	Cross-reactivity (%)
Human Omentin 1	100
Human Vaspin	0
Human FTO	0
Human Endothelial Lipase	0
Human ADRP	0
Human Adiponectin, globular form	0
Human CTRP9	0
Human CTRP3	0
Human Periostin/OSF-2	0
Human PBEF/Visfatin	0
Human FGF-21	0
Human RBP-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 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 the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 5-10 minutes on the plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read at 450nm within 3 min.