HUMAN OMENTIN 1 ELISA KIT

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

PURCHASE INFORMATION:

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
Pretreatment	Require
Dilution Factor	5 (Optimal dilutions should be determined by each laboratory for each application)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2°C - 8°C

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

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INTRODUCTION

Human OMENTIN 1 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human OMENTIN 1 in cell culture supernates or tissues, serum, and plasma. It contains recombinant human OMENTIN 1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human OMENTIN 1. Results obtained with naturally occurring OMENTIN 1 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human OMENTIN 1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for OMENTIN 1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any OMENTIN 1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for OMENTIN 1 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of OMENTIN 1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS 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 preservatives; lyophilized.	**020-01-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrate of biotinylated antibody against OMENTIN 1 with preservatives; 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 uL/vial, 100-fold concentrated solution of Streptavidin conjugated to HRP	**SAHRP**	**1 vial**
Sample Buffer – 20mL of 0.1% SDS in solution	**STB01**	**1 bottle**
Dilution Buffer – 60mL of buffered protein based solution with preservatives	**DB06**	**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 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should

be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months. Reconstituted Positive Control should be prepared and used within a few days.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

*All samples must be treated before being added to the microplate. Standard and Positive Control **DO NOT NEED** Pretreatment.*

1. Add 50 µL of sample to 200 µl of **Sample Buffer** in a polypropylene tube. **Note:** This pretreatment dilution may require optimization.
2. Vortex gently and incubate for 30 minutes at room temperature. Assay immediately and discard any excess pretreated samples.

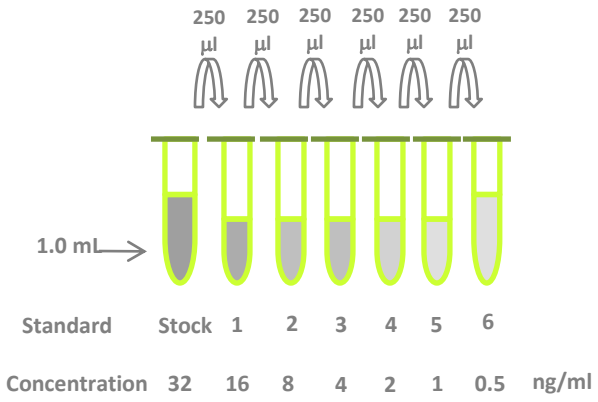
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.
***Note:** Pretest is required to optimize the dilution factor needed for samples. If samples are undetectable, add TCEP or DTT to a final concentration of 10mM to the treated sample.

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 Wash Buffer.

OMENTIN 1 Standard - Refer to vial label for reconstitution volume. 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. **Note:** Positive Control should be prepared and used within a few days.

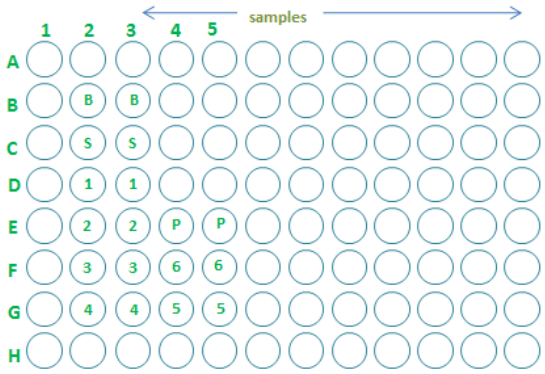
Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (C2, C3 to G2, G3 and F4, F5 to G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-6 minutes at room temperature. **Protect from light.**
11. 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.
12. Determine the optical density of each well within 15 minutes, using a micro-plate 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 curve fit. As an alternative, construct a standard curve by plotting

the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human OMENTIN 1.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of OMENTIN 1 was 125 pg/mL.

TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.055)
0.5	0.056
1	0.108
2	0.164
4	0.339
8	0.665
16	1.370
32	2.583

***Lot No.:**

**** Positive Control: 1.0– 3.0 ng/mL**

SPECIFICITY

This assay recognizes both natural and recombinant human OMENTIN 1. The factors listed below were prepared at 500 ng/mL in Dilution Buffer, and assayed for cross reactivity.

ADIPOKINES	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, samples, 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 3-6 minutes on the plate shaker. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 minutes