Version 14-04/24

User's Manual

Human Omentin-1 ELISA

REF **DEIA4741**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human Omentin-1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human omentin-1.

General Description

Omentin (intelectin-1, intestinal lactoferin receptor, endothelial lectin HL-1, galactofuranosebinding lectin) is newly identified secretory protein that is highly and selectively expressed in visceral adipose tissue relative to subcutaneous adipose tissue (adipokine). The mature omentin is secretory glycoprotein consisting of 295 amino acids and 1-linked oligosacharides, and its basic structural unit is a 120-kDa homotrimer in which 40-kDa polypeptides are bridged by disulfide bonds. Omentin has been identified in other tissue at lower expression levels such are Paneth cells, endothelial cells, and visceral adipose stromal-vascular cells.

A homolog of omentin has been identified that shares 83% amino acid identity with omentin and was referred to as omentin 2. The two omentin genes, omentin 1 and omentin 2, are localized adjacent to each other in chromosomal region, which has been previously linked to type 2 diabetes in several populations.

To determine the impact of obesity-dependent insulin resistance on the regulation of two omentin isoforms, gene expression and plasma levels were measured in lean, overweight, and obese subjects. Omentin-1 was shown to be the major circulating isoform in human plasma. Lean subjects had significantly higher omentin-1 plasma levels than obese and overweight subjects. In addition, higher plasma omentin-1 levels were detected in women compared with men. Plasma omentin-1 levels were inversely correlated with BMI, waist circumference, leptin levels, and insulin resistance as measured by homeostasis model assessment and positively correlated with adiponectin and HDL levels. In summary, decreased omentin-1 levels are associated with increasing obesity and insulin resistance.

An independent experiment reported that the addition of recombinant omentin-1 in vitro did not affect basal glucose uptake but did enhance insulin-stimulated glucose uptake in both subcutaneous and omental human adipocytes. Omentin-1 increased Akt phosphorylation in the absence and presence of insulin and may regulate insulin action.

A recent study of women with the polycystic ovary syndrome (PCOS) found significantly reduced omentin-1 mRNA expression and protein levels in adipose tissue in overweight PCOS women. In addition, significantly lower plasma omentin-1 levels were detected in these women.

Principles of Testing

In the Human Omentin-1 ELISA, standards, quality controls and samples are incubated at 37°C in microplate wells pre-coated with polyclonal anti-human omentin-1 antibody. After 120 minutes incubation and washing, biotin labelled polyclonal anti-human omentin-1 antibody is added and incubated at 37°C with captured omentin-1 for 30 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation at 37°C and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of omentin-1. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of

unknown samples are determined using this standard curve.

Reagents And Materials Provided

Antibody Coated Microtiter Strips, ready to use, 96 wells

Biotin Labelled Antibody Conc. (50×), concentrated, 0.28 ml

Streptavidin-HRP Conjugate, ready to use, 13 ml

Master Standard, lyophilized, 2 vials

Quality Control HIGH, lyophilized, 2 vials

Quality Control LOW, lyophilized, 2 vials

Biotin-Ab Diluent, ready to use, 13 ml

Dilution Buffer, ready to use, 20 ml

Wash Solution Conc. (10×), concentrated, 100 ml

Substrate Solution, ready to use, 13 ml

Stop Solution, ready to use, 13 ml

Materials Required But Not Supplied

1. Deionized (distilled) water
2. Test tubes for diluting samples
3. Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
4. Precision pipettes to deliver 2-1000 µl with disposable tips
5. Multichannel pipette to deliver 100 µl with disposable tips
6. Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
7. Vortex mixer
8. 37°C Incubator
9. Microplate washer (optional). (Manual washing is possible but not preferable.)
10. Microplate reader with 450±10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
11. Software package facilitating data generation and analysis (optional)

Storage

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Specimen Collection And Preparation

The kit measures human omentin-1 in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Preparation of serum or plasma samples:

Dilute samples just prior to performing the assay 40× with Dilution Buffer, e.g. 4 µl of sample+156 µl of Dilution Buffer for singlets, or preferably 6 µl of sample + 234 µl of Dilution Buffer for duplicates.

Mix well (not to foam). Vortex is recommended.

1. Stability and storage:

Serum and plasma samples should be stored at -20°C, or preferably at -70°C for long-term storage.

Do not store the diluted samples.

However, no decline in concentration of omentin-1 was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	-20°C	532	428	524	612
	2-8°C, 1 day	544	420	480	584
	2-8°C, 7 days	536	364	544	584
2	-20°C	599	438	592	644
	2-8°C, 1 day	604	448	544	649
	2-8°C, 7 days	600	368	597	668
3	-20°C	880	608	776	916
	2-8°C, 1 day	921	604	776	912
	2-8°C, 7 days	864	536	780	908

2. Effect of Freezing/Thawing

No decline was observed in concentration of human omentin-1 in serum and plasma samples after repeated (5×) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

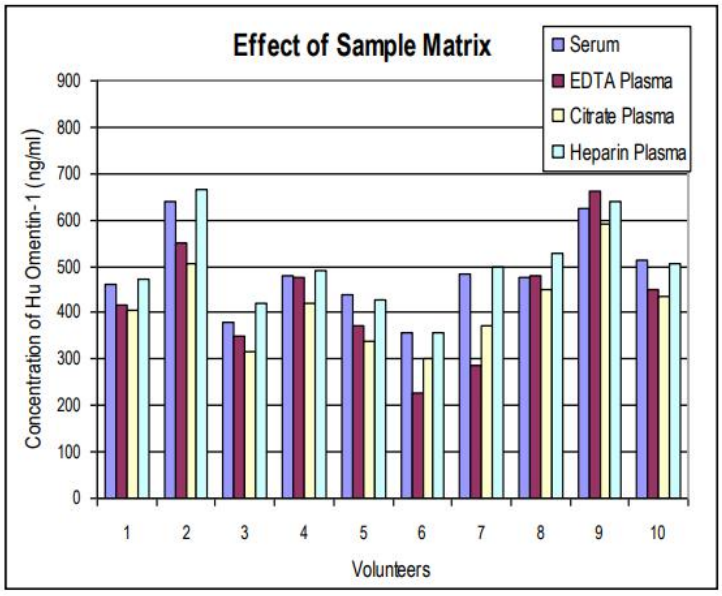
Sample	Number of f/t cycles	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	1x	440	436	440	526
	3x	439	401	439	451
	5x	452	412	452	446
2	1x	510	525	510	580
	3x	519	515	519	593
	5x	522	568	522	581
3	1x	447	360	447	497
	3x	434	245	434	444
	5x	419	302	419	448

3. Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

Omentin-1 levels measured using Human Omentin-1 ELISA from10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
1	462	416	404	473
2	641	549	507	666
3	381	348	316	422
4	480	475	422	489
5	440	371	339	429
6	357	227	300	356
7	484	286	373	499
8	475	482	449	529
9	624	661	593	640
10	514	450	437	507
Mean (ng/ml)	**486**	**426**	**414**	**501**
Mean Plasma/Serum (%)	-	**87.6**	**85.2**	**103.1**
Coefficient of determination R^2	-	**0.72**	**0.86**	**0.95**



Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

Plate Preparation

Example of a work sheet

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 64	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 32	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 16	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 8	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 4	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 2	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	QC HIGH	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC LOW	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Reagent Preparation

1. **Human Omentin-1 Master Standard**

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of Standard!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the omentin-1 in the stock solution is **64 ng/ml**.

Prepare set of standards using **Dilution Buffer** as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	64 ng/ml
250 ml of stock	250 µl	32 ng/ml
250 ml of 32 ng/ml	250 µl	16 ng/ml
250 ml of 16 ng/ml	250 µl	8 ng/ml
250 ml of 8 ng/ml	250 µl	4 ng/ml
250 ml of 4 ng/ml	250 µl	2 ng/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage: Do not store the Standard stock solution and set of standards.

2. **Quality Controls HIGH, LOW**

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!

Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage: Do not store the reconstituted Quality Controls.

Note: Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or

pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

3. **Biotin Labelled Antibody Conc. (50×)**

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (50×) with 49 parts Biotin-Ab Diluent. Example: 20 µl of Biotin Labelled Antibody Concentrate (50×) + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage: Opened Biotin Labelled Antibody Concentrate (50×) is stable 3 months when stored at 2-8°C.

Do not store the diluted Biotin Labelled Antibody solution.

4. **Wash Solution Conc. (10×)**

Dilute Wash Solution Concentrate (10×) ten-fold in distilled water to prepare a 1x working solution.

Example: 100 ml of Wash Solution Concentrate (10×) + 900 ml of distilled water for use of all 96-wells.

Stability and storage: The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

Assay Procedure

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure for example of work sheet.
2. Incubate the plate at **37°C** for **2 hours** without shaking.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at **37°C** for **30 minutes** without shaking.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at **37°C** for **30 minutes** without shaking.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well on a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550-650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

    Note: If some samples and standards have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values

measured at 405 nm, is used to determine omentin-1 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

Calculation

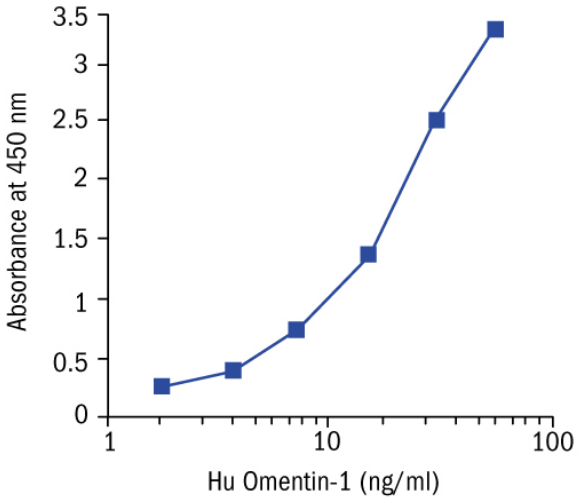
Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of omentin-1 ng/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 10 ng/ml (from standard curve) x 40 (dilution factor) = 400 ng/ml.

The recombinant human omentin-1 is used as the Standard. The recombinant human omentin-1 (AA 19-298), produced in *E.coli*, is 32.7 kDa protein containing 280 amino acid residues of the human omentin-1 and 14 extra AA.

Typical Standard Curve

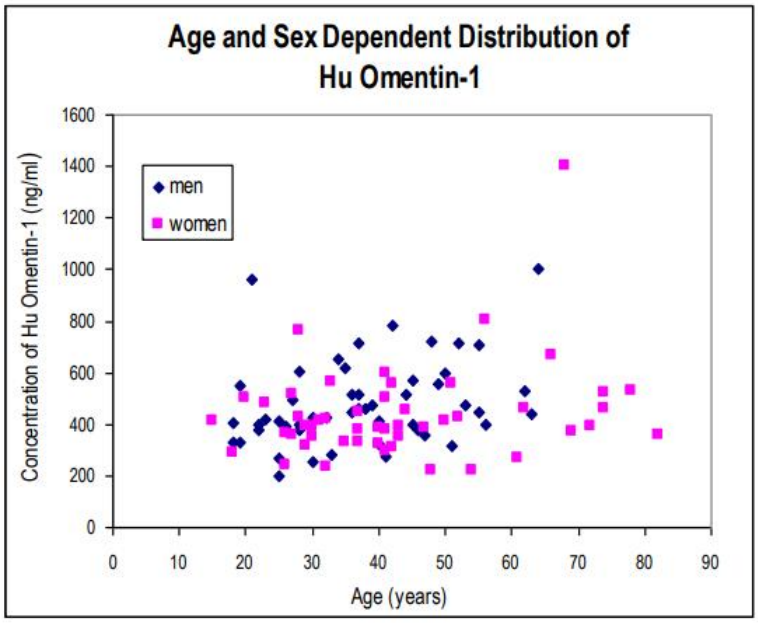


Reference Values

The following results were obtained when serum samples from 100 unselected donors (50 men + 50 women) 15-82 years old were assayed with the Human Omentin-1 ELISA.

1. **Age dependent distribution of omentin-1**

Sex	Age (years)	n	Mean	SD	Min.	Max.
			Omentin-1 ng/ml			
Men	18-29	16	433	165	200	960
	30-39	13	480	125	252	712
	40-49	11	481	157	272	784
	50-64	10	562	189	318	1 000
Women	15-29	12	421	131	242	764
	30-37	10	385	81	236	560
	40-48	13	396	103	220	600
	50-82	15	534	280	221	1 399



2. **Reference range**

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological references ranges for omentin-1 levels with the assay.

Performance Characteristics

It is intended for research use only.

The total assay time is less than 3.5 hours.

The kit measures omentin-1 in serum and plasma (EDTA, citrate, heparin).

Assay format is 96 wells.

Quality Controls are human serum based. No animal sera are used.

Standard is recombinant protein based.

Components of the kit are provided ready to use, concentrated or lyophilized.

Detection Range

2-64 ng/ml

Detection Limit

0.5 ng/ml

Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank: $A_{blank} + 3 \times SD_{blank}$) is calculated from the real human omentin-1 values in wells and is 0.5 ng/ml.

Note: Dilution Buffer is pipetted into blank wells.

Specificity

The antibodies used in this ELISA are specific for human omentin-1. Sera of several mammalian species were measured in the assay. See results below.

Mammalian serum sample	Observed crossreactivity
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	644	-	-
	2x	321	322	99.7
	4x	167	161	103.7
	8x	83	81	102.5
2	-	394	-	-
	2x	208	197	105.6
	4x	109	98	111.2
	8x	50	49	102.0

Recovery

Serum samples were spiked with different amounts of omentin-1 and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	301	-	-
	888	941	94.4
	583	621	93.9
	425	461	92.2
2	158	-	-
	772	798	96.7
	455	478	95.2
	306	318	96.2

Reproducibility

1. **Intra-assay (Within-Run) (n=8)**

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	322	13.6	4.1
2	478	15.2	3.2

2. **Inter-assay (Run-to-Run) (n=8)**

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	409	19.8	4.8
2	480	21.0	4.4

Precautions

1. **For professional use only**
2. Wear gloves and laboratory coats when handling immunodiagnostic materials
3. Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
4. This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
5. This kit contains components of animal origin. These materials should be handled as potentially infectious
6. Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
7. The materials must not be pipetted by mouth

Trouble Shooting

1. **Weak signal in all wells**

   Possible explanations:

   a. Omission of a reagent or a step

   b. Improper preparation or storage of a reagent

c. Assay performed before reagents were allowed to come to room temperature

d. Improper wavelength when reading absorbance

2. **High signal and background in all wells**

   Possible explanations:

   a. Improper or inadequate washing

   b. Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution

   c. Incubation temperature over 40°C

3. **High coefficient of variation (CV)**

   Possible explanations:

   a.Improper or inadequate washing

   b. Improper mixing Standards, Quality Controls or samples

Limitations

1. Reagents with different lot numbers should not be mixed
2. Use thoroughly clean glassware
3. Use deionized (distilled) water, stored in clean containers
4. Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
5. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
6. Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
7. Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements