

Human Omentin-1
96 Well Plate Assay
Cat. # EZH0MNTN1-29K

# HUMAN OMENTIN-1 ELISA KIT 96-Well Plate (Cat. # EZH0MNTN1-29K)

I.	Intended Use	2
II.	Principles of Assay	2
III.	Reagents Supplied	3
IV.	Storage and Stability	5
٧.	Reagent Precautions	5
VI.	Materials Required But Not Provided	7
VII.	Sample Collection and Storage	7
VIII.	Reagent Preparation	8
IX.	Assay Procedure	9
Χ.	Microtiter Plate Arrangement	12
XI.	Calculations	13
XII.	Interpretation	13
XIII.	Graph of Typical Reference Curve	14
XIV.	Assay Characteristics	15
XV.	Quality Controls	17
XVI.	Troubleshooting Guide	18
(VII.	Replacement Reagents	18
VIII.	Ordering Information	19

## HUMAN OMENTIN-1 ELISA KIT 96-Well Plate (Cat# EZH0MNTN1-29K)

#### I. INTENDED USE

This kit is used for the non-radioactive quantification of Omentin-1 in Human serum/plasma. One kit is sufficient to measure 38 unknown samples in duplicate. *This kit is for Research Use Only. Not for Use in Diagnostic Procedures.* 

#### II. PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based on: 1) capture of Omentin-1 molecules in the sample by anti-Omentin IgG and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titered amount of anchor antibodies, 2) and the simultaneous binding of a second biotinylated antibody to Omentin-1, 3) wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 4) wash-away of free enzyme, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetra-methylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450nm, corrected from the absorbency at 590nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured Omentin-1 in the unknown sample, the concentration of Omentin-1 can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Omentin-1.

#### III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

#### 1. Microtiter Plate

Coated with pre-titered anchor antibodies.

Quantity: 1 Strip Plate

Preparation: Ready to use.

Note: Unused strips should be resealed in the foil pouch with the

dessicant provided and stored at 2-8 °C.

#### 2. Adhesive Plate Sealer

Quantity: 2 sheets

Preparation: Ready to use.

#### 3. 10X HRP Wash Buffer Concentrate

10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.

Quantity: 2 bottles containing 50 ml each

Preparation: Dilute 1:10 with distilled or de-ionized water.

#### 4. Human Omentin-1 Standard

Human Omentin-1 reference standard in buffer, 200 ng/mL.

Quantity: 1 bottle

Preparation: Ready to use. Dilute according to instructions in §VIII. A.

### 5. Quality Controls 1 and 2

QC solutions containing Human Omentin-1 at two different levels.

Quantity: One vial each, 0.5 mL per vial.

Preparation: Ready to use.

#### 6. Matrix Solution

Processed Serum Matrix with 0.08% Sodium Azide, lyophilized.

Quantity: 1 bottle

Preparation: Hydrate with 1 mL distilled or de-ionized water

## 7. Assay Buffer

0.05 M phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.05 % Triton X-100, 0.08% Sodium Azide, and 0.1% BSA

Quantity: 15 mL/vial

Preparation: Ready to use.

## III. REAGENTS SUPPLIED (continued)

# 8. Human Omentin Capture Antibody

Pre-titered capture antibody solution in buffer

Quantity: 3 mL/vial

Preparation: Mix 1:1 with Human Omentin Detection Antibody before use

according to § VIII. B.

# 9. Human Omentin Detection Antibody

Pre-titered detection antibody solution in buffer

Quantity: 3 mL/vial

Preparation: Mix 1:1 with Human Omentin Capture Antibody before use

according to § VIII. B.

## 10. Enzyme Solution

Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in buffer.

Quantity: 12 mL/vial

Preparation: Ready to use

## 11. Substrate

3, 3',5,5'-tetramethylbenzidine in buffer.

Quantity: 12 mL/vial

Preparation: Ready to use. Minimize the exposure to light.

## 12. Stop Solution

0.3 M HCI

Quantity: 12 mL/vial

Preparation: Ready to use.

[Caution: Corrosive Solution]

#### IV. STORAGE AND STABILITY

Recommended storage for kit components is 2-8°C.

All components are shipped and stored at 2-8°C. Once opened, liquid standards and controls can be stored up to 30 days at 2-8°C. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers

#### V. REAGENT PRECAUTIONS

#### 1. Sodium Azide

Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

## 2. Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

# 3. Blood components

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Note: See Full Labels of Hazardous components on next page.

Full labels of hazardous components in this kit:

-ull labels of hazardou Ingredient, Cat #		Full Label	
Human Omentin Capture Antibody	E1029-C		Warning. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Human Omentin Detection Antibody	E1029-D		Warning. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Human Omentin-1 Quality Controls 1 & 2	E6029-K		Warning. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Human Omentin Standard	E8029-K	<b>!</b>	Warning. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Assay Buffer	EABGR	<b>!</b>	Warning. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Matrix Solution	EMTX-PS4		Danger. Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Stop Solution	ET-TMB		Warning. May be corrosive to metals.
10X HRP Wash Buffer Concentrate	EWB-HRP		Warning. May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

#### VI. MATERIALS REQUIRED BUT NOT PROVIDED

- 1. Pipettes and pipette tips: 10 μL -20 μL or 20 μL -100 μL
- 2. Multi-channel Pipettes and pipette tips: 5 -50 μL and 50 -300 μL
- 3. Buffer and Reagent Reservoirs
- Vortex Mixer
- De-ionized Water
- Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm
- 7. Orbital Microtiter Plate Shaker
- 8. Absorbent Paper or Cloth

### VII. SAMPLE COLLECTION AND STORAGE

- 1. To prepare serum samples, whole blood is directly drawn into a Vacutainer® serum tube that contains no anticoagulant. Mix well and let blood clot at room temperature for 30 min.
- 2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2°C.
- 3. Transfer and store serum samples in separate tubes. Date and identify each sample.
- 4. Use freshly prepared serum or store samples in aliquots at  $\leq$  -20°C for later use. Avoid repeated freeze/thaw cycles.
- 5. To prepare plasma samples, whole blood should be collected into Vacutainer  $^{\tiny (8)}$  EDTA-plasma tubes, placed on ice and centrifuged immediately at 2,000 to 3,000 x g for 15 min at 4  $\pm$  2°C. Transfer and store plasma samples as outlined for serum samples.
- 6. Because human Omentin-1 molecule contains a fibrinogen-β like motif, the presence of high amount of plasma fibrinogen may cause higher Omentin-1 results in plasma than serum samples even though the cross-reactivity to fibrinogen appears minimal. Therefore, serum samples are more preferable.
- 7. Avoid using samples with gross hemolysis or lipemia.

#### VIII. REAGENT PREPARATION

## A. Standard Preparation

 Label six tubes with the additional concentrations of standards to be prepared: 2 ng/mL, 4 ng/mL, 10 ng/mL, 20 ng/mL, 40 ng/mL and 100 ng/mL. Add Assay Buffer to each of the six tubes according to the volumes outlined in the chart below. Dilute the 200 ng/mL standard stock according to the chart below. Vortex each tube briefly to ensure complete mixing.

Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of standard should be stored in small aliquots at 2-8°C. Avoid multiple freeze/thaw cycles.

Concentration of Standards	Volume of 200 ng/mL Stock to Add	Volume of Assay Buffer to Add
2 ng/mL	0.010 mL	0.990 mL
4 ng/mL	0.020 mL	0.980 mL
10 ng/mL	0.050 mL	0.950 mL
20 ng/mL	0.100 mL	0.900 mL
40 ng/mL	0.200 mL	0.800 mL
100 ng/mL	0.500 mL	0.500 mL
200 ng/mL		

## B. Preparation of Capture and Detection Antibody Mixture

Prior to use, combine the entire contents of Human Omentin Capture Antibody (3 mL) and Human Omentin Detection Antibody (3 mL), or at a 1:1 ratio, and invert to mix thoroughly. Any remaining combined Capture/Detection Antibody should be discarded after use and should not be re-used.

## C. Preparation of Matrix Solution

Prior to use, reconstitute each bottle with 1 mL distilled or de-ionized water.

#### IX. HUMAN OMENTIN ELISA ASSAY PROCEDURE

Pre-warm all reagents to room temperature immediately before setting up the assay. Dilute all samples 4X with Assay Buffer in small microfuge tubes, e.g. add 60  $\mu$ L Assay Buffer to 20  $\mu$ L of sample and mix well. Do not store diluted sample for later use. [Alternatively, 5  $\mu$ L undiluted samples can be used in each sample well and the Assay Buffer in sample wells increased from 30  $\mu$ L to 45  $\mu$ L (see steps 8 and 4). This can save time and labor while achieving same results, if and only if pipetting 5  $\mu$ L sample is precise and accurate.]

- Dilute the 10X concentrated HRP Wash Buffer 10 fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.
- 2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C. Assemble the strips in an empty plate holder and fill each well with 300 μl diluted Wash Buffer. Decant Wash Buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 2 additional times. Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.
- 3. Add 20  $\mu$ L Matrix Solution to Blank, Standards and Quality Control wells (refer to § X. for suggested well orientations).
- 4. Add 30 μL Assay Buffer to each of the Blank and sample wells.
- 5. Add 10 μL Assay Buffer to each of the Standard and Quality Control wells.
- 6. Add in duplicate 20 μL Omentin-1 Standards in the order of ascending concentrations to the appropriate wells.
- 7. Add in duplicate 20 μL QC1 and 20 μL QC2 to the appropriate wells.
- 8. Add sequentially 20  $\mu$ L of the unknown samples in duplicate to the remaining wells.
- 9. Transfer the Antibody Solution Mixture (i.e.,1:1 mixture of capture and detection antibody) to a buffer or reagent reservoir and add 50 µL to each well with a multi-channel pipette.
- 10. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.
- 11. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.

## IX. HUMAN OMENTIN ELISA ASSAY PROCEDURE (CONTINUED)

- 12. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
- 13. Add 100 μL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 min on the micro-titer plate shaker.
- 14. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid.
- 15. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
- 16. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 5 to 20 minutes. Blue color should be formed in wells of the Omentin-1 standards with intensity proportional to increasing concentrations of Omentin-1.

**Note:** Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

17. Remove sealer and add 100 µL Stop Solution [CAUTION: CORROSIVE SOLUTION] and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest Omentin-1 standard should be approximately 1.5 – 2.2, or not to exceed the capability of the plate reader used.

# Assay Procedure for Human OMENTIN-1 ELISA Kit (Cat. # EZH0MNTN1-29K)

	Step 1	Step 2	Step 3	Step 4-5	Step 6-8	Step 9	Step 10-12	Step 13	Step 14-15	Step 16	}	Step '	17
Well #	er.		Matrix Solution	Assay Buffer	Standards/QCs/ Samples	Capture/ Detection Ab. Mixture		Enzyme Solution		Substrate		Stop Solution	
A1, B1	d wat	<u>s</u>	20 µL	30 µL									
C1, D1	Dilute both bottles of 10X HRP Wash Buffer with 900 mL de-ionized water.	Wash plate 3X with 300 µL diluted HRP Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	20 µL	10 µL	20 μL of 2 ng/mL Standard	50 μL	ture.	100 μL	ature .	100 μL	- 20 minutes at Room Temperature.	100 μL	
E1, F1	0 mL de	3X with 300 µL diluted HRP Wash Buffer. buffer by tapping smartly on absorbent to	20 µL	10 µL	20 μL of 4 ng/mL Standard		Seal, Agitate, Incubate 2 hours at Room Temperature. Wash 3X with 300 µL Wash Buffer.		Seal, Agitate, Incubate 30 minutes at Room Temperature Wash 6X with 300 µL Wash Buffer.		n Temp		0 nm.
G1, H1	with 90	HRP W	20 μL	10 μL	20 μL of 10 ng/mL Standard		Room T sh Buff		e, Incubate 30 minutes at Room Te Wash 6X with 300 µL Wash Buffer.		at Roor		Read Absorbance at 450 nm and 590 nm.
A2, B2	Buffer	diluted ng sma	20 μL	10 μL	20 μL of 20 ng/mL Standard		ours at l pL Wa		nutes af ) µL Wa		ninutes		450 ոm
C2, D2	P Wash	300 µL y tappi	20 µL	10 µL	20 μL of 40 ng/mL Standard		ate 2 hc with 300		e 30 mii vith 300		5 - 20 n		ance at
E2, F2	10X HR	3X with buffer b	20 μL	10 μL	20 μL of 100 ng/mL Standard		e, Incub ash 3X		Incubat		cubate		Absorb
G2, H2	ttles of	Wash plate ve residual	20 μL	10 μL	20 µL of 200 ng/mL Standard		Agitate Wa		gitate, l Wa		Seal, Agitate, Incubate 5		Read
A3, B3	th bo	Was ove re	20 µL	10 µL	20 µL of QC 1		Seal,		eal, A		al, Ag		
C3, D3	ıte bo	Rem	20 µL	10 µL	20 μL of QC 2	<b>↓</b>		<b>+</b>	S	<b>\</b>	Se		
E3, F3	Dill			30 µL	20 µL of diluted Sample 1								
G3, H3 Etc.				30 µL	20 µL of diluted Sample 2		_						

# X. MICROTITER PLATE ARRANGEMENT

**Human OMENTIN-1 ELISA** 

	1	2	3	4	5	6	7	8	9	10	11	12
А	Blank	20 ng/mL	QC1	Etc.								
В	Blank	20 ng/mL	QC1	Etc.								
С	2 ng/mL	40 ng/mL	QC2									
D	2 ng/mL	40 ng/mL	QC2									
Е	4 ng/mL	100 ng/mL	Sample 1									
F	4 ng/mL	100 ng/mL	Sample 1									
G	10 ng/mL	200 ng/mL	Sample 2									
Н	10 ng/mL	200 ng/mL	Sample 2									

#### XI. CALCULATIONS

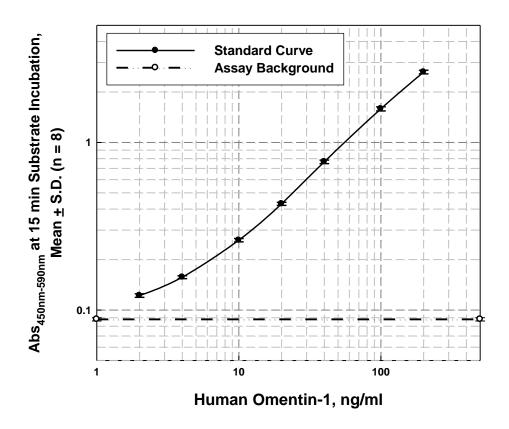
The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function, then multiplied by the sample dilution factor 4.

**Note:** When sample (4X diluted) volumes assayed differ from 20  $\mu$ L, an appropriate mathematical adjustment must be made to accommodate for the extra dilution factor (e.g., if 10  $\mu$ L of diluted sample is used, then calculated data must be multiplied by 2, then by 4). When sample volume assayed is less than 20  $\mu$ L, compensate the volume deficit with Matrix Solution.

## XII. INTERPRETATION

- The assay will be considered accepted when all Quality Control values fall within the calculated QC range. If any QCs fall outside of the control range, review results with a supervisor.
- 2. If the difference between duplicate results of a sample is >15% CV, repeat the sample.
- 3. The theoretical minimal detecting concentration of this assay is 0.23 ng/mL Omentin-1 (20  $\mu$ L 4X diluted sample size).
- 4. The appropriate range of this assay is 2 ng/mL to 200 ng/mL Omentin-1 (20  $\mu$ L 4X diluted sample size). Any result greater than 200 ng/mL in a 20  $\mu$ L 4X diluted sample should be further diluted using Matrix Solution and the assay repeated until the results fall within range.

# XIII. GRAPH OF TYPICAL REFERENCE CURVE



Typical Standard Curve, not to be used to calculate data.

#### XIV. ASSAY CHARACTERISTICS

## A. Analytical Sensitivity

The lowest level of Omentin-1 that can be detected by this assay is 0.23 ng/mL when using a 20  $\mu$ L 4X diluted sample size, as derived from Statistical Ligand Immunoassay Analysis of multiple assays (n = 8) calculating the mean plus 2 standard deviations of the minimal detectable concentrations.

# B. Specificity

Human Omentin-1	100.0%
Human Omentin-2	104.9%
Rat Omentin-1	1.7%
Human Plasma Fibrinogen	0.0035%

#### C. Precision

## **Intra- and Inter-Assay Variations (n = 6)**

Sample		OMENTIN-1 Intra-assay (ng/mL) CV (%)		Inter-assay CV (%)
	1	83.2	1.28	3.62
Plasma	2	70.9	1.10	11.00
	3	117.3	2.18	2.91
	1	19.3	2.82	6.90
Serum	2	16.5	2.90	6.29
	3	52.9	3.56	4.78

Fasting human serum/plasma aliquots are assayed for Omentin-1 by ELISA. Intraassay variations were calculated from results of 6 duplicate determinations in one assay. Inter-assay variations were calculated from results of 6 separate assays with duplicate samples in each assay.

# XIV. ASSAY CHARACTERISTICS (continued)

## D. Analyte Spike Recovery Rate

Sample		Basal OMENTIN -1	_	IENTIN-1 ng/mL)		IENTIN-1 ng/mL)	+ OMENTIN-1 (100 ng/mL)		
		ng/mL	ng/m L	Recover y	ng/m L	Recover y	ng/m L	Recover y	
	1	13.8	22.2	84.0 %	47.2	83.5 %	93.2	79.4 %	
	2	8.0	18.2	102.0 %	48.0	100.0 %	102.8	94.8 %	
	3	28.0	37.8	98.0 %	67.6	99.0 %	125.0	97.0 %	
٤	4	26.2	36.6	104.0 %	65.0	97.0 %	119.2	93.0 %	
Serum	% Recover y Rate (Mean ± S.D., n = 4)	100 %	97.7 %	ú ± 9.02 %	95.0 %	6 ± 7.44 %	91.0 % ± 8.16 %		
	1	5.3	17.7	124.0 %	43.7	96.0 %	96.0	90.7 %	
	2	7.6	17.2	96.0 %	46.8	98.0 %	105.0	97.4 %	
	3	12.0	21.9	99.0 %	52.8	102.0 %	119.4	107.4 %	
ma	4	13.7	23.1	94.0 %	52.9	98.0 %	115.1	101.4 %	
Plasma	% Recover y Rate (Mean ± S.D., n = 4)	100 %	103.3 %	% ± 14.0 %	98.5 %	6 ± 2.52 %	99.2 %	6 ± 7.01 %	

Fasting Human serum and plasma samples are diluted 4-fold with Assay Buffer and divided into several aliquots. Then the indicated concentrations of Human Omentin-1 are spiked into diluted sample aliquots for measurement of recovery rate. Omentin-1 levels reported in the Table are not corrected for dilution factors. The recovery rate = [(Observed Omentin-1 concentration after spike - Basal Omentin-1 level) / spiked Omentin-1 concentration] x 100%.

# XIV. ASSAY CHARACTERISTICS (continued)

## E. Linearity of Sample Dilution

		Sample Volume Assayed								
	Sample	20 μL	15 µL		1	Ι0 μL		5 μL		
	Sample	ng/mL	ng/ mL	Expected	ng/ mL	Expected	ng/ mL	Expected		
	1	120.8	91.0	100.4 %	63.0	104.3 %	33.4	110.6 %		
ے ا	2	86.8	65.2	100.2 %	45.2	104.2 %	24.6	113.4 %		
בַּ	3	71.0	52.2	98.0 %	36.8	103.7 %	19.8	111.6 %		
Serum	4	45.6	35.6	104.1 %	24.2	106.1 %	12.8	112.3 %		
"	% Expected (Mean ± S.D., n = 4)	100 % 100.7 % ± 2.53 %		104.6 % ± 1.05 %		112.0 % ± 1.18 %				
	1	406.8	302. 8	99.2 %	207. 2	101.9 %	109. 2	107.4 %		
a	2	259.6	194. 4	99.8 %	132. 0	101.7 %	68.0	104.8 %		
Plasma	3	155.6	117. 6	100.8 %	36.8	107.5 %	19.8	121.3 %		
	4	115.2	91.2	105.6 %	65.6	113.9 %	37.2	129.2 %		
	% Expected (Mean ± S.D., n = 4)	100 %	101.4	% ± 2.91 %	106.3	5 % ± 5.77 %	115.7	' % ± 11.6 %		

Fasting human serum and plasma samples are spiked with Omentin-1 to various levels and then diluted 4-fold with Assay Buffer for Omentin-1 ELISA. All diluted samples were assayed at 20  $\mu$ L, 15  $\mu$ L, 10  $\mu$ L and 5  $\mu$ L per well and samples volumes less than 20  $\mu$ L are compensated with Matrix Solution. Measured Omentin-1 levels are corrected for various dilution factors and then divided by levels found at 20  $\mu$ L sample size to obtain the % of expected values.

### **XV. QUALITY CONTROLS**

The ranges for each Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website <a href="emdmillipore.com">emdmillipore.com</a> using the catalog number as the keyword.

#### XVI. TROUBLESHOOTING GUIDE

- 1. To obtain reliable and reproducible results, the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- 2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- 3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started, all steps should be completed with precise timing and without interruption.
- 4. Avoid cross-contamination of any reagents or samples to be used in the assay.
- 5. Make sure all reagents and samples are added to the bottom of each well.
- 6. Careful and complete mixing of solutions in the wells is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- 7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- 8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with HRP Wash Buffer or 3) overexposure to light after substrate has been added.

### XVII. REPLACEMENT REAGENTS

Reagents Microtiter Plates 10X HRP Wash Buffer Concentrate (50 ml)	<b>Cat. #</b> EPDAR EWB-HRP
Human Omentin-1 Standard	E8029-K
Human Omentin-1 Quality Controls 1 and 2	E6029-K
Matrix Solution	EMTX-PS4
Assay Buffer	EABGR
Human Omentin Capture Antibody	E1029-C
Human Omentin Detection Antibody	E1029-D
Enzyme Solution	EHRP-4
Substrate	ESS-TMB3
Stop Solution	ET-TMB

### XVIII. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

### **Conditions of Sale**

For Research Use Only. Not for Use in Diagnostic Procedures.

# **Safety Data Sheets (SDS)**

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at <a href="mailto:emdmillipore.com/msds">emdmillipore.com/msds</a>.