Human High Molecular Weight (HMW) Adiponectin ELISA Kit

96-Well Plate Assay

Cat. # EZHMWAN-65K

HUMAN HIGH MOLECULAR WEIGHT (HMW) ADIPONECTIN ELISA KIT
96-Well Plate

Cat. # EZHMWAN-65K

TABLE OF CONTENTS **PAGE**

EMD Millipore Corporation • 3050 Spruce Street • St. Louis, Missouri 63103 USA
Toll Free U.S: 1-800 645-5476 • Fax: 1-800-645-5439
emdmillipore.com

I. INTENDED USE

This Human High Molecular Weight (HMW) Adiponectin ELISA kit is used for the non-radioactive quantification of Human HMW Adiponectin in serum, plasma, and adipocyte extracts or culture media samples with a simple sample pretreatment. One kit is sufficient to measure 37 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, sequentially, on: 1) capture of Human HMW Adiponectin molecules from pretreated samples to the wells of a microtiter plate coated with a monoclonal mouse anti-adiponectin antibody, 2) washing of unbound materials from samples, 3) binding of a second biotinylated polyclonal goat anti-adiponectin antibody to the captured molecules, 4) washing of unbound materials from samples, 5) binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies, 6) washing of excess free enzyme conjugates, and 7) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm – 590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured Human HMW Adiponectin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Human HMW Adiponectin.

III. REAGENTS SUPPLIED

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

Note: Store all reagents at 2-8°C

Reagents Supplied	Catalog Number	Volume	Quantity
Microtiter Plate with 2 plate sealers	EP65	---------	1 plate 2 sealers
Sample Preparation Plates (2) with 2 plate sealers	ESD-PLATE	---------	2 plates 2 sealers
10X Wash Buffer	EWB-HRP	50 mL	2 bottles
Human HMW Adiponectin Standard	E8065-K	lyophilized	1 vial
Human HMW Adiponectin Quality Controls 1 and 2	E6065-K	lyophilized	2 vials
Sample Digestion Solution	ESDS-1	50 µL	1 tube
Sample Digestion Buffer	EDGB-1	5 mL	1 bottle
Sample Dilution Buffer	ESDB-1	500 µL	1 tube
10X Assay Buffer	EAB-10XP	50 mL	1 bottle
Assay Running Buffer	EARB-7	13 mL	1 bottle
Human HMW Adiponectin Detection Antibody	E1065-K	12 mL	1 bottle
Enzyme Solution	EHRP	12 mL	1 bottle
Substrate Solution	ESS-TMB	12 mL	1 bottle
Stop Solution	ET-TMB	12 mL	1 bottle

IV. STORAGE AND STABILITY

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

All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. 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

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.

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

A. Full Labels of Hazardous component(s):

Ingredient, Cat #		Full Label	
10X Wash Buffer	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Human HMW Adiponectin Standard	E8065-K		**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.
Human HMW Adiponectin Quality Controls 1 and 2	E6065-K		**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.
Sample Digestion Solution	ESDS-1		**Danger.** May cause allergy or asthma symptoms or breathing difficulties if inhaled. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
Sample Digestion Buffer	EDGB-1		**Danger.** Causes serious eye damage. 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.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Multi-channel Pipettes and pipette tips: 5-50 µL and 50-300 µL
2. Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Vortex Mixer
6. De-ionized water
7. Microtiter Plate Reader capable of reading absorbency at 450 nm
8. Orbital Microtiter Plate Shaker
9. Absorbent Paper or Cloth
10. 37°C Incubator

VII. SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and proceed to sample digestion preparation immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple >3 freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Serum samples should be digested prior to assay according to provided sample preparation or alternate procedure provided.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and proceed to sample digestion preparation immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple >3 freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Plasma samples should be digested prior to assay according to provided sample preparation or alternate sample preparation procedure provided.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

VIII. SAMPLE DIGESTION PROCEDURES

The sample digestion procedures provided in this protocol should be carried out just prior to assay setup.

Prepare 1X Assay Buffer (Cat# EAB-10XP), by adding 40 mL of EAB-10XP to 360 mL of deionized water. Mix well.

The catalog numbers for Sample Digestion Solution (ESDS-1), Sample Digestion Buffer (EDGB-1) and Sample Dilution Buffer (ESDB-1) are similar. Take care in using the appropriate catalog number during the digestion procedure.

Option A) Sample Digestion – Sample Preparation Plate

1. Centrifuge samples for 5 minutes at 10,000xg.
2. Label Sample Preparation Plates 1 and 2.
3. Add 85 µL of Sample Digestion Buffer (EDGB-1) to the wells of Plate 1 for each sample to be digested.
4. Add 10 µL of each sample to wells containing Sample Digestion Buffer.
5. Seal plate and shake vigorously for 5 minutes on an orbital microtiter plate shaker (~700-800 rpm). **Note: It is important to shake samples vigorously to avoid high CVs. Avoid splashing on the sealer to prevent cross-contamination.**
6. During the 5 minute incubation, add 30 µL of Sample Digestion Solution (ESDS-1) to 270 µL of Sample Digestion Buffer in a polypropylene microfuge tube. Vortex well.
7. Remove plate from shaker, add 5 µL of diluted Sample Digestion Solution to sample wells. (Samples are diluted 1:10)
8. Seal plate and shake vigorously for 5 minutes on an orbital microtiter plate shaker (~700-800 rpm). **Note: It is important to shake samples vigorously to avoid high CVs. Avoid splashing on the sealer to prevent cross-contamination.**
9. Incubate plate at 37°C for 2 hours.
10. Remove plate from incubator and shake vigorously for 5 minutes on an orbital microtiter plate shaker (~700-800 rpm). **Note: It is important to shake samples vigorously to avoid high CVs. Avoid splashing on the sealer to prevent cross-contamination.**
11. While shaking, prepare 1X Sample Dilution Buffer by adding 450 µL 10X Sample Dilution Buffer (ESDB-1) to 4.05 mL 1X Assay Buffer (EAB-10XP). Mix well.
12. Add 95 µL 1X Sample Dilution Buffer to the corresponding sample wells of Sample Preparation Plate #2.
13. Transfer 5 µL of digested samples from Plate 1 to Plate 2. (Samples are diluted 1:200)

14. Seal plate and shake vigorously for 5 minutes on an orbital microtiter plate shaker (~700-800 rpm). **Note: It is important to shake samples vigorously to avoid high CVs. Avoid splashing on the sealer to prevent cross-contamination.**
15. Assay immediately.

    Note: 1:200 digested samples may be stored at -20°C for future use.

Option B) Sample Digestion – Polypropylene Microcentrifuge Tubes

1. Centrifuge samples for 5 minutes at 10,000xg.
2. Add 85 µL of Sample Digestion Buffer (EDGB-1) to microcentrifuge tubes for each sample to be digested.
3. Add 10 µL of each sample to the tubes containing Sample Digestion Buffer. Vortex well. **Note: It is important to vortex samples vigorously to avoid high CVs.**
4. Prepare diluted Sample Digestion Solution (ESDS-1) by adding 30 µL to 270 µL of Sample Digestion Buffer in a polypropylene microfuge tube. Vortex well.
5. Add 5 µL of diluted Sample Digestion Solution to each sample tube and vortex well. (Samples are diluted 1:10) **Note: It is important to vortex samples vigorously to avoid high CVs.**
6. Incubate sample tubes at 37°C for 2 hours.
7. Prepare 1X Sample Dilution Buffer by adding 450 µL 10X Sample Dilution Buffer (ESDB-1) to 4.05 mL 1X Assay Buffer (EAB-10XP). Mix well.
8. After the 2 hour incubation, remove tubes from incubator and vortex well. **Note: It is important to vortex samples vigorously to avoid high CVs.**
9. Add 95 µL 1X Sample Dilution Buffer to a second set of microcentrifuge tubes for each digested sample.
10. Transfer 5 µL of digested samples to the second set of tubes for each sample. Vortex well. **Note: It is important to vortex samples vigorously to avoid high CVs.** (Samples are diluted 1:200)
11. Assay immediately.

    Note: 1:200 digested samples may be stored at -20°C for future use.

IX. REAGENT PREPARATION

A. Human HMW Adiponectin Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human HMW Adiponectin Standard with 0.25 mL distilled or de-ionized water. Invert and mix gently, let sit for 5 minutes then mix well.

2. Label seven polypropylene microfuge tubes as 1, 2, 3, 4, 5, 6 and 7. Add 100 µL of Assay Running Buffer to each of the seven tubes. Prepare serial dilutions by adding 100 µL of the reconstituted standard to Tube 7, mix well and transfer 100 µL of Tube 7 to Tube 6, mix well and transfer 100 µL of Tube 6 to Tube 5, mix well and transfer 100 µL of Tube 5 to Tube 4, mix well and transfer 100 µL of the Tube 4 to Tube 3, mix well and transfer 100 µL of Tube 3 to Tube 2, mix well and transfer 100 µL of Tube 2 to Tube 1, mix well. The 0 ng/mL standard (Background) will be Assay Running Buffer.

   Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤-20°C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration (ng/mL)
Reconstituted standard	250 µL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Running Buffer to Add	Volume of Standard to Add	Standard Concentration (ng/mL)
Tube 7	100 µL	100 µL of reconstituted standard	X/2
Tube 6	100 µL	100 µL of Tube 7	X/4
Tube 5	100 µL	100 µL of Tube 6	X/8
Tube 4	100 µL	100 µL of Tube 5	X/16
Tube 3	100 µL	100 µL of Tube 4	X/32
Tube 2	100 µL	100 µL of Tube 3	X/64
Tube 1	100 µL	100 µL of Tube 2	X/128

IX. REAGENT PREPARATION (continued)

B. Human HMW Adiponectin Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Human HMW Adiponectin Quality Control 1 and Quality Control 2 with 0.25 mL distilled or de-ionized water and gently invert to ensure complete hydration. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ -20°C. Avoid further freeze/thaw cycles.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 100 mL of 10X Wash Buffer (two bottles) with 900 mL deionized water. Store unused portion at 2-8°C for up to one month.

X. HUMAN HMW ADIPONECTIN ELISA ASSAY PROCEDURE

Warm all reagents to room temperature before setting up the assay.

1. 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. Add 300 µL diluted Wash Buffer to each well of the plate. Decant Wash Buffer and remove the residual volume by inverting the plate and tapping it smartly onto absorbent towels several times. Repeat wash 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.

2. Add 90 µL Assay Running Buffer (EARB-7) to all wells.

3. Add in duplicate 10 µL Assay Running Buffer to each of the Blank wells.

4. Add in duplicate 10 µL Standards or Controls to the appropriate wells.

5. Add in duplicate 10 µL of pre-treated samples to the appropriate wells.

6. 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.

7. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

8. Add 100 µL Detection Antibody to each well. Re-cover plate with sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400-500 rpm.

9. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

10. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

X. HUMAN HMW ADIPONECTIN ELISA ASSAY PROCEDURE (continued)

11. Remove sealer, decant reagents from the plate and tap plate to remove the residual volume. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

12. 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 Human HMW Adiponectin standards with intensity proportional to increasing concentrations of Human HMW Adiponectin.

    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.

13. Remove sealer and add 100 µL Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and gently 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 450nm and 590nm 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 Human HMW Adiponectin standard should be approximately 2.0 - 3.0, or not to exceed the capability of the plate reader used.

    Note: Pre-treated samples are diluted 1:200. Final results should be multiplied by a dilution factor of 200.

    When sample volumes assayed differ from 10 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 5 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10 µL, compensate for the volume deficit with 1X Assay Buffer (EAB-10XP).

Assay Procedure for Human HMW Adiponectin ELISA Kit (Cat. # EZHMWAN-65K)

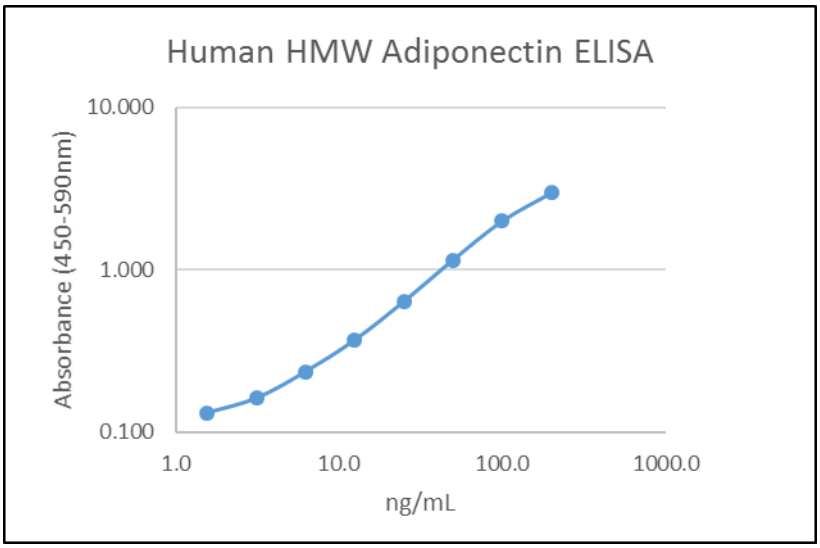
	Step 1	Step 2	Step 3-5	Step 6-7	Step 8	Step 9	Step 10	Step 11	Step 12-13			
Well #	**Wash plate 3X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.**	Assay Running Buffer	Standards/ QCs/ Samples	**Seal, Agitate, Incubate 2 hrs at Room Temperature. Wash 3X with 300 µL Wash Buffer.**	Detection Antibody	**Seal, Agitate, Incubate 1 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer.**	Enzyme Solution	**Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 3X with 300 µL Wash Buffer.**	Substrate	**Seal, Agitate, Incubate 5 – 20 minutes at Room Temperature.**	Stop	**Read Absorbance at 450 nm and 590 nm.**
A1, B1		90 µL	10 µL Assay Running Buffer		100 µL		100 µL		100 µL		100 µL	
C1, D1			10 µL of Tube 1									
E1, F1			10 µL of Tube 2									
G1, H1			10 µL of Tube 3									
A2, B2			10 µL of Tube 4									
C2, D2			10 µL of Tube 5									
E2, F2			10 µL of Tube 6									
G2, H2			10 µL of Tube 7									
A3, B3			10 µL Reconstituted Standard									
C3, D3			10 µL of QC1									
E3, F3			10 µL of QC2									
G3, H3 Etc.			10 µL of Sample									

XI. MICROTITER PLATE ARRANGEMENT

Human HMW Adiponectin ELISA

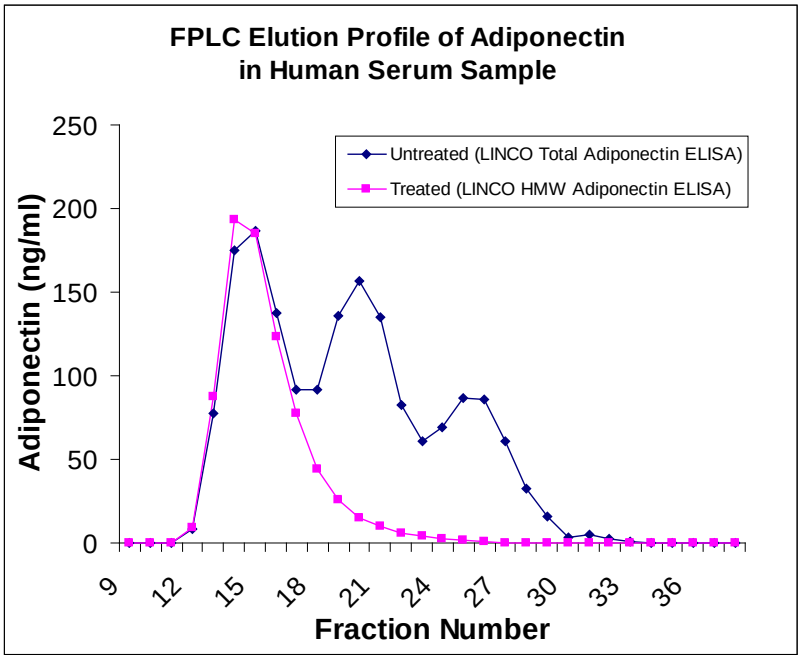
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 4	Reconstituted Standard	etc								
B	Blank	Tube 4	Reconstituted Standard	etc								
C	Tube 1	Tube 5	QC1									
D	Tube 1	Tube 5	QC1									
E	Tube 2	Tube 6	QC2									
F	Tube 2	Tube 6	QC2									
G	Tube 3	Tube 7	Sample 1									
H	Tube 3	Tube7	Sample 1									

XII. GRAPH OF TYPICAL REFERENCE CURVE



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

XIII. GRAPH OF FPLC PROFILE



XIV. ASSAY CHARACTERISTICS

A. Sensitivity

The Minimum Detectable Concentration (MinDC) of HMW Adiponectin is 1.5 ng/mL calculated by using MILLIPLEX® Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. This reported value is the mean plus 2 standard deviations of the MinDC of multiple assays (n= 8).

B. Specificity

With sample pretreatment, this assay measures HMW Adiponectin. The antibody pair used in this assay is specific to Adiponectin and does not significantly cross-react to the following molecules/hormones tested:

Adipsin	Non-detectable
Chemerin	Non-detectable
HGF	Non-detectable
IL-1β	Non-detectable
IL-6	Non-detectable
IL-8	Non-detectable
Insulin	Non-detectable
Leptin	Non-detectable
Lipocalin-2/NGAL	Non-detectable
MCP-1	Non-detectable
NGF	Non-detectable
Omentin	Non-detectable
PAI-1	Non-detectable
Resistin	Non-detectable
TNFα	Non-detectable

XIV. ASSAY CHARACTERISTICS (continued)

C. Precision

Intra-Assay Variation

	Mean Adiponectin Levels (ng/mL)	Intra-Assay %CV
1	7.8	< 5
2	42.4	< 5

Inter-Assay Variation

	Mean Adiponectin Levels (ng/mL)	Inter-Assay %CV
1	9.2	<15
2	42.9	<15

The assay variations of EMD Millipore's Human HMW Adiponectin ELISA kit was studied on two samples at two levels on the HMW Adiponectin standard curve. The mean intra-assay variation was calculated from results of eight determinations of the indicated samples. The mean inter-assay variations of each sample were calculated from results of 8 separate assays with duplicate samples in each assay.

XIV. ASSAY CHARACTERISTICS (continued)

D. Spike Recovery of HMW Adiponectin in Assay Samples

Sample	Adiponectin Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	Recovery
1	6.25	51.7	50.4	98%
	12.5	57.9	56.9	98%
	25.0	70.4	71.3	101%
2	6.25	33.9	35.2	104%
	12.5	40.2	41.1	102%
	25.0	52.7	53.7	102%
3	6.25	17.7	19.3	109%
	12.5	23.9	25.5	106%
	25.0	36.4	40.0	110%
4	6.25	40.5	43.5	107%
	12.5	46.8	48.7	104%
	25.0	59.3	65.2	110%
5	6.25	60.2	61.9	103%
	12.5	66.5	68.5	103%
	25.0	79.0	79.8	101%
Average				104%

Varying amounts of Human Adiponectin were added to individual human serum and plasma samples and the resulting Adiponectin content of each sample was assayed by Human HMW Adiponectin ELISA. The recovery = [(observed Adiponectin / (spiked Adiponectin concentration + basal Adiponectin level)] x 100%.

XIV. ASSAY CHARACTERISTICS (continued)

E. Linearity of Sample Dilution

Sample	Volume (µL)	Expected (ng/mL)	Observed (ng/mL)	Expected
1	10	52.3	52.3	
	5	26.2	26.8	103%
	2.5	13.1	12.5	96%
	1.25	6.54	5.3	81%
2	10	27.4	27.4	
	5	13.7	13.1	95%
	2.5	6.85	5.78	84%
	1.25	3.43	2.61	76%
3	10	13.7	13.7	
	5	6.85	6.52	95%
	2.5	3.43	3.00	88%
	1.25	1.71	1.66	97%
4	10	9.62	9.62	
	5	4.81	4.99	104%
	2.5	2.41	2.49	104%
	1.25	1.21	<1.28	
5	10	57.6	57.6	
	5	28.8	32.4	112%
	2.5	14.4	15.6	108%
	1.25	7.20	7.02	98%
Average				96%

Five human serum and plasma samples with the indicated sample volumes were assayed. Required amounts of 1X Assay Buffer were added to compensate for lost volumes below 10 µL. The resulting dilution factors of 1, 2, 4 and 8 representing 10 µL, 5 µL, 2.5 µL and 1.25 µL sample volumes assayed, respectively, were applied in the calculation of observed Adiponectin concentrations. % expected = (observed/expected) x 100%

XV. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website emdmillipore.com.

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 well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High signal 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 Wash Buffer or 3.) overexposure to light after substrate has been added.

XVII. REPLACEMENT REAGENTS

Reagents	Cat. #
Human HMW Adiponectin ELISA Plate	EP65
Sample Preparation Plates	ESD-PLATE
10X HRP Wash Buffer Concentrate	EWB-HRP
Human HMW Adiponectin Standard	E8065-K
Human HMW Adiponectin Quality Controls 1 and 2	E6065-K
Sample Digestion Solution	ESDS-1
Sample Digestion Buffer	EDGB-1
Sample Dilution Buffer	ESDB-1
10X Assay Buffer	EAB-10XP
Assay Running Buffer	EARB-7
Human HMW Adiponectin Detection Antibody	E1065-K
Enzyme Solution	EHRP
Substrate Solution	ESS-TMB
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 emdmillipore.com/msds.