Human Chemerin
96-Well Plate Assay
Cat. # EZHCMRN-57K

HUMAN CHEMERIN ELISA KIT
96-Well Plate (EZHCMRN-57K)

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Human Chemerin ELISA KIT
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I. INTENDED USE

This Human Chemerin ELISA kit is used for the non-radioactive quantification of Human Chemerin in serum, plasma, tissue extract and cell culture samples. One kit is sufficient to measure 38 unknown samples in duplicate. ***This kit is for research purpose only.***

II. PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of Human Chemerin molecules from samples to the wells of a microtiter plate coated by a pre-titered amount of anti-Human Chemerin antibody, 2) wash away of unbound materials from samples, 3) binding of a biotinylated anti-human Chemerin antibody to the captured molecules, 4) wash away of unbound materials from samples, 5) conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 6) wash away of 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 absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured Human Chemerin 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 Chemerin.

III. REAGENTS SUPPLIED

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

A. Human Chemerin ELISA Plate
Coated with anti-Human Chemerin antibody
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.

B. Adhesive Plate Sealer
Quantity: 2 sheets
Preparation: Ready to Use

C. 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 deionized water.

III. REAGENTS SUPPLIED (continued)

D. Human Chemerin Standard
Human Chemerin, lyophilized.
Quantity: 0.5 mL/vial upon hydration
Preparation: Reconstitute with 0.5 mL distilled or deionized water.

E. Human Chemerin Quality Controls 1 and 2
One vial each, lyophilized, containing Human chemerin at two different levels..
Quantity: 0.5 mL/vial upon hydration
Preparation: Reconstitute with 0.5 mL distilled or deionized water.

F. Assay Running Buffer
0.05 Phosphosaline, pH 7.6 containing 0.025M EDTA, 0.08% Sodium Azide, 1.0% BSA
Quantity: 2 bottles containing 10 mL each
Preparation: Ready to Use

G. Serum Matrix
Serum matrix solution, liquid
Quantity: 0.5mL/vial
Preparation: Add 0.5 mL of Assay Running Buffer into glass vial and invert and mix gently. The matrix solution is 1:2 diluted

H. Human Chemerin Detection Antibody
Pre-titered Biotinylated anti-human Chemerin Antibody
Quantity: 12 mL
Preparation: Ready to Use

I. Enzyme Solution
Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer
Quantity: 12 mL
Preparation: Ready to Use

J. Substrate (Light sensitive, avoid unnecessary exposure to light)
3, 3', 5, 5'-tetramethylbenzidine in buffer
Quantity: 12 mL
Preparation: Ready to Use.

K. Stop Solution (Caution: Corrosive Solution)
0.3 M HCl
Quantity: 12 mL
Preparation: Ready to Use

IV. STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C. For longer storage (> 2 weeks), freeze Wash Buffer, Assay Running Buffer, Chemerin Standards, Quality Controls and reconstituted Standards and Controls at ≤ –20°C. Minimize repeated freeze and thaw of the Chemerin Standards and Quality Controls. 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

A. Sodium Azide

Sodium azide has been added to certain reagents as a preservative. Although the concentrations are low, sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

B. 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.

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
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 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 centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.

   Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2°C.

   Transfer and store serum samples in separate tubes. Date and identify each sample.

   Use freshly prepared serum or aliquot and store samples at ≤ –20°C for later use. For long-term storage, keep at -70 °C. Avoid freeze/thaw cycles.

2. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/mL and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.

3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.

4. Avoid using samples with gross hemolysis or lipemia.

VIII.SAMPLE PREPARATION

1. Normal human serum or plasma samples need 1:5 dilution using Assay Running Buffer (mix 50 µL sample with 200µl Assay Running Buffer) prior to assay.

2. Tissue extracts or cell culture media samples may require dilution. Dilutions should be performed using the Assay Running Buffer provided.

IX. REAGENT PREPARATION

A. Human Chemerin Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human Chemerin Standard with 0.5 mL distilled or deionized water into the glass vial to give a concentration described in the analysis sheet. Invert and mix gently and let sit for 5 minutes then mix well.

2 Label six tubes, 1, 2, 3, 4, 5 and 6. Add 100 µL Assay Running Buffer to each of the six tubes. Perform 2 time serial dilutions by adding 100 µL of the standard to Tube 1, mix well and transfer 100 µL from Tube 1 to Tube 2, mix well and transfer 100 µL from Tube 2 to Tube 3, mix well and transfer 100 µL from Tube 3 to Tube 4, mix well and transfer 100 µL from Tube 4 to Tube 5, mix well and transfer 100uL from Tube 5 to Tube 6 and mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Concentration ng/mL
0.5 mL	0	X (refer to analysis sheet For exact concentration)

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

B. Human Chemerin Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Human Chemerin Quality Control 1 and Quality Control 2 with 0.5 mL distilled or deionized water into the glass vials. Invert and mix gently, let sit for 5 minutes then mix well.

C. Serum Matrix Preparation

Use care in opening matrix vial. Using a pipette, add 0.5 mL of Assay Running Buffer into glass vial. Invert and mix gently. The matrix solution is 1:2 diluted.

X. ASSAY PROCEDURE

Pre-warm all reagents to room temperature prior to setting up the assay.

1. Dilute the 10X concentrated Wash Buffer 10 fold by mixing the entire contents of each bottle of wash buffer concentrate with 450mL deionized or distilled water (dilute both buffer bottles with 900mL deionized or 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 wash each well 3 times with 300µl of diluted wash buffer per wash. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, use a gentle wash program for all washing steps described in this protocol.**

3. Add in duplicate, 80 µL Assay Running Buffer to the blank wells.

4. Add in duplicate, 60 µL Assay Running Buffer to the Standard wells, QC1 and QC2 wells.

5. Add in duplicate, 80 µL Assay Running Buffer to sample wells.

6. Add in duplicate, 20 µL 1:2 diluted matrix solution to blank wells, Standard wells, QC1 and QC2 wells.

7. Add in duplicate, 20 µL Human Chemerin Standards in the order of ascending concentration to the appropriate wells. Add in duplicate, 20 µL QC1 and 20 µL QC2 to the appropriate wells. Add 20µl unknown samples (1:5 dilution with Assay Running Buffer is required for serum or plasma samples) in duplicate sequentially to the remaining wells. **For best result all additions should be completed within 30 minutes.**

8. Cover the plate with plate sealer and incubate at room temperature for 1.5 hours on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

9. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

10. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

11. Add 100 µL Detection Antibody to all wells. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

X. ASSAY PROCEDURE (continued)

12. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

13. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

14. 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.

15. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.

16. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

17. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for 5 to 20 minutes (A longer development time may be needed if using a plate washer). Blue color should be formed in wells of Human Chemerin standards with intensity proportional to increasing concentrations of Chemerin.

18. 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. 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 Chemerin standard should be approximately 2.-3.2, or not to exceed the capability of the plate reader used.

Assay Procedure for Human Chemerin ELISA kit (EZHCMRN-57K)

	Step 1	Step 2	Step 3-5	Step 6	Step 7	Step 8-10	Step 11	Step 11-13	Step 14	Step 14-16	Step 17	Step 17	Step 18	Step 18
Well #	**Dilute each of 10X Wash Buffer with 450 mL Deionized Water.**	**Wash 3X with 300 µl Wash Buffer Remove residual buffer by tapping smartly on absorbent towels**	Assay Running Buffer	1:2 diluted matrix	Buffer/ Standards Controls/ Samples	**Seal, Agitate, Incubate 1.5 hour at Room Temperature. Wash 3X with 300 µl Wash Buffer**	Detection Ab	**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 Solution	**Read Absorbance at 450 nm and 590 nm.**
A1, A2			80 µl	20 µl	----		100 µl		100 µl		100 µl		100 µl	
A3, A4			60 µl		20 µl of Tube 6 Std									
A5, A6					20 µl of Tube 5 Std									
A7, A8					20 µl of Tube 4 Std									
A9, A10					20 µl of Tube 3 Std									
A11, A12					20 µl of Tube 2 Std									
B1, B2					20 µl of Tube 1 Std									
B3, B4					20 µl reconstituted Standard									
B5, B6					20 µl of QC1									
B7, B8					20 µl of QC2									
B9, B10			80 µl	0 µl	20 µl of Sample									
B11, B12			80 µl	0 µl	20 µl of Sample									

XI. MICROTITER PLATE ARRANGEMENT

Human Chemerin ELISA

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

XII. CALCULATIONS

The dose-response curve of this assay fits best to a 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. The serum or plasma sample data need to be multiplied by 5 (dilution factor) to obtain the final sample concentration.

Note: When sample volumes assayed differ from 20 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 4). When sample volume assayed is less than 20 µL, compensate the volume deficit with Assay Running Buffer.

XIII. INTERPRETATION

A. Acceptance Criteria

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.

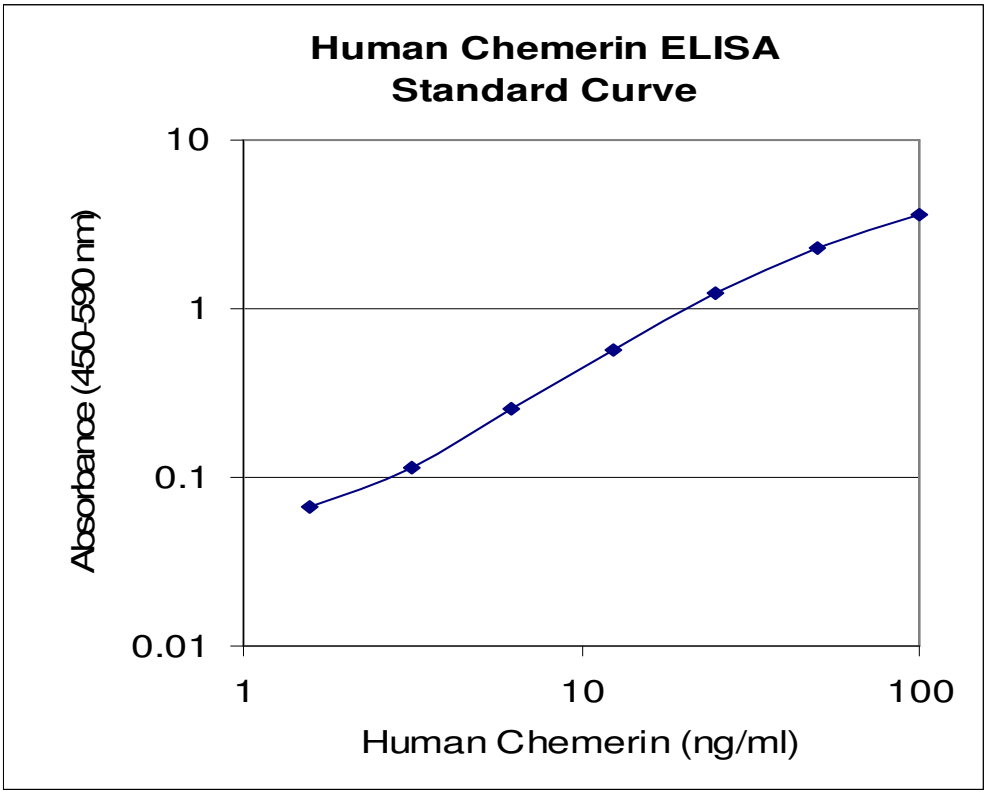
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.

3. The limit of sensitivity of this assay is 1.56 ng/mL Human Chemerin (20 µL sample size).

4. The appropriate range of this assay is 1.560ng/mL to 100 ng/mL Human Chemerin (20 µL sample size). Any result greater than 100 ng/mL in a 20 µL sample should be diluted using Assay Running Buffer, and the assay repeated until the results fall within range. Tissue/cell extracts or cell culture media samples greater than 100 ng/mL in a 20 µL sample should be diluted in Assay Running Buffer.

XIV. STANDARD CURVE

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



XV. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Human Chemerin that can be detected by this assay is 1.56 ng/mL when using a 20 µL sample size.

B. Specificity

The antibody pair used in this assay is specific to human chemerin and has no significant cross-reactivity with mouse chemerin or other human adipokines.

XV. ASSAY CHARACTERISTICS (continued)

C. Precision

Intra-Assay Variation

Sample No.	Mean Chemerin Levels (ng/mL)	Intra-Assay %CV
1	10.6	5%
2	40.4	5%

Inter-Assay Variation

Sample No.	Mean Chemerin Levels (ng/mL)	Inter-Assay %CV
1	11.5	4%
2	40.5	6%

The assay variations of Human Chemerin ELISA kits were studied on two samples at two levels on the Chemerin 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 was calculated from results of four separate assays with duplicate samples in each assay.

D. Recovery

Spike & Recovery of Human Chemerin in Serum

Sample No.	Chemerin Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	% of Recovery
1	0	15.6	15.6	
	6.25	21.9	20.2	92%
	12.5	28.1	26.6	95%
	25	40.6	42.9	106%
2	0	12.4	12.4	
	6.25	18.7	19.0	102%
	12.5	24.9	22.7	91%
	25	37.4	41.0	110%
3	0	11.5	11.5	
	6.25	17.8	16.4	92%
	12.5	24.0	22.4	93%
	25	36.5	37.2	102%

Varying amounts of Human Chemerin were added to three Human serum samples and the Chemerin concentration was determined. The % of recovery = observed Chemerin concentrations/expected Chemerin concentrations x 100%.

XV. ASSAY CHARACTERISTICS (continued)

E. Linearity

Effect of Serum Dilution

Sample No.	Volume Sampled (µL)	Expected (ng/mL)	Observed (ng/mL)	% Of Expected
1	20	19.9		
	10	9.95	10.6	107%
	5	4.98	4.9	98%
	2.5	2.49	1.9	76%
2	20	16.3		
	10	8.15	9	110%
	5	4.08	5.2	128%
	2.5	2.04	1.8	88%
3	20	17.7		
	10	8.85	9.1	103%
	5	4.43	4.4	99%
	2.5	2.21	1.6	72%
4	20	16.5		
	10	8.25	8.6	104%
	5	4.13	3.9	95%
	2.5	--	--	--

Four Human serum samples with the indicated sample volumes were assayed. Required amounts of Assay Running Buffer were added to compensate for lost volumes below 20 µL. The resulting dilution factors of neat, 2, 4, and 8 representing 20 µL, 10 µL, 5 µL, and 2.5 µL sample volumes assayed, respectively, were applied in the calculation of observed Chemerin concentrations. % expected = observed/expected x 100%.

XVI. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com/bmia .

XVII. 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 bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. 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 Wash Buffer or 3) overexposure to light after substrate has been added.

XVIII. REPLACEMENT REAGENTS

Reagents	Cat. #
Human Chemerin ELISA Plate	EP57
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
Human Chemerin Standards	E8057-K
Human Chemerin Quality Controls 1 and 2	E6057-K
Assay Running Buffer	EARB
Serum Matrix	EMTX-PS5
Chemerin Detection Antibody	E1056
Enzyme Solution	EHRP
Substrate	ESS-TMB2
Stop Solution	ET-TMB

XIX. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

TELEPHONE ORDERS:
Toll Free US (800) MILLIPORE

FAX ORDERS: (636) 441-8050

MAIL ORDERS: Millipore
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:
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B. Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals. All products are intended for *in vitro* use only.

C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore Research products may be ordered by fax or phone. See Section A above for details on ordering.