

HUMAN CHEMERIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN CHEMERIN CONCENTRATIONS
IN CELL CULTURE SUPERNATES, SERUM,
AND PLASMA



PURCHASE INFORMATION:

ELISA NAME	HUMAN CHEMERIN ELISA
Catalog No.	SK00171-01
Lot No.	
Formulation	96 T
Standard range	31.25-2000 pg/mL
Sensitivity	15.6 pg/mL
Sample Volume	100 µl
Dilution Factor	100 for serum or plasma (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma, urine, Cell Culture Supernates
Specificity	Human CHEMERIN only
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.

Order Contact:
AVISCIERA BIOSCIENCE
 2348 Walsh Ave. Suite C
 Santa Clara, CA 95051
 USA
 Tel: (408) 982 0300
 Fax: (408) 982 0301
 Info@AvisceraBioscience.com
 www.AvisceraBioscience.com

INTRODUCTION

Human CHEMERIN immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human CHEMERIN in urine, cell culture supernatants, serum, and plasma. It contains recombinant CHEMERIN and antibodies raised against this protein. It has been shown to accurately quantify recombinant CHEMERIN. Results obtained with naturally occurring CHEMERIN 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 CHEMERIN.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CHEMERIN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CHEMERIN present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for CHEMERIN 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 CHEMERIN 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
Chemerin Microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against CHEMERIN.	171-01-01	1 plate
CHEMERIN Standard – 2000 pg/vial of recombinant CHEMERIN in a buffered protein base with preservatives; lyophilized.	171-01-02	1 vial
Detection Concentrate – 105 µL/vial, 100-fold concentrate of Biotinylated antibody against CHEMERIN with preservatives; lyophilized.	171-01-03	1 vial
Positive Control – one vial of recombinant CHEMERIN, lyophilized	171-01-04	1 vial
Streptavidin-HRP Conjugate – 60 uL/vial, 200-fold concentrated solution of Streptavidin conjugated to HRP with preservatives	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	DB06	2 bottles
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
Substrate Solution – 11 mL of TMB substrate solution	SS01	1 bottle
Stop Solution – 11 mL of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one

month. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 12 months. Reconstituted Positive Control should be prepared and used immediately.

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

Serum and plasma samples may need a 100-fold dilution. A suggested 100-fold dilution is 5 µL sample + 495 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

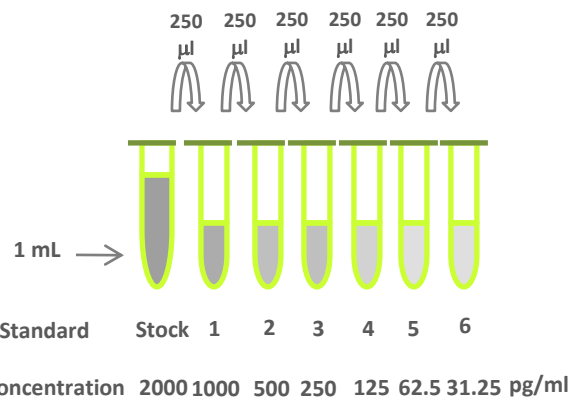
REAGENT PREPARATION

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.

CHEMERIN Standard - Refer to vial label for reconstitution volume. Reconstitute the **CHEMERIN** Standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/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 (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	2000 pg/ml
# 1	250µl of stock	250µl	1000 pg/ml
# 2	250µl of 1	250µl	500 pg/ml
# 3	250µl of 2	250µl	250 pg/ml
# 4	250µl of 3	250µl	125 pg/ml
# 5	250µl of 4	250µl	62.5 pg/ml
# 6	250µl of 5	250µl	31.25 pg/ml



Positive Control – Reconstitute the **Positive Control** with 1.0 mL Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

Detection Concentrate – Reconstitute the Detection Concentrate with 105 µL of Dilution Buffer to

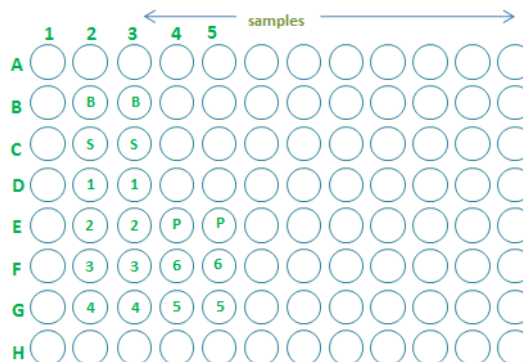
produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 μ L of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 μ L of 200-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 microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 μ L of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 μ L 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 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 **1x Wash Buffer** (300 μ L) 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 μ L of **Detection working solution** to each well. Cover with plate sealer. Incubate for 2 hours on plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μ L of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 μ L of **Substrate Solution** to each well. Incubate for 4-6 minutes at room temperature. **Protect from light.**
11. Add 100 μ L 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 microplate 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 CHEMERIN 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 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 CHEMERIN.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CHEMERIN was 10 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 (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.053)
31.25	0.013
62.5	0.027
125	0.074
250	0.183
500	0.451
1000	0.989
2000	1.970

LINEARITY

To assess the linearity of the assay, pooled research **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
200X	1019.430	203886	100
400X	481.854	192741.6	95

To assess the linearity of the assay, pooled research **plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
100X	1050.495	105049.5	100
200X	469.699	93939.8	90

SPECIFICITY

	Cross-reactivity
Human Chemerin	100%
Mouse Chemerin	0
Human Resistin	0
Human Adiponectin	0
Human FGF-21	0
Human Visfatin	0
Human Omentin 1	0

REFERENCES:

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2. Röss C, et al. Effect of bariatric surgery on circulating chemerin levels. Eur J Clin Invest. 2010 Jan 25. [Epub ahead of print]
3. Kukla M, et al. Serum chemerin and vaspin in non-alcoholic fatty liver disease. Scand J Gastroenterol. 2010;45(2):235-42.

SUMMARY OF ASSAY PROCEDURE

