HUMAN CHEMERIN ELISA KIT

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

PURCHASE INFORMATION:

NAME	HUMAN CHEMERIN ELISA
Catalog No.	SK00171-02
Lot No.	
Formulation	96 T
Standard range	0.78 – 50 ng/mL
Sensitivity	500 pg/ml
Sample Volume	100 µl
Dilution Factor	40 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma, cell culture
Specificity	Human Chemerin only
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	4 °C

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Order Contact:
AVISCERA BIOSCIENCE INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
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 cell culture supernates, serum, and plasma. It contains recombinant human CHEMERIN and antibodies raised against this protein. It has been shown to accurately quantify recombinant human 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 human CHEMERIN.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal 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 polyclonal 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
Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against CHEMERIN.	171-02-01	1 plate
CHEMERIN Standard – 200 ng/vial of recombinant human CHEMERINin a buffered protein base with preservatives; lyophilized.	171-02-02	1 vial
Detection Antibody Concentrate – 120µL/vial, 100-fold Concentrate of Biotinylated polyclonal antibody against CHEMERINwith preservatives; lyophilized.	171-02-03	1 vial
Positive Control - 10-fold Concentrate of recombinant human CHEMERIN, lyophilized	171-02-04	1 vial
Streptavidin-HRP Conjugate - 120 uL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
HRP Diluent Solution – 12mL/vial of buffered protein based solution with preservatives	DB06C	1 vial
Dilution Buffer - 60mL/vial of buffered protein based solution with preservatives	DB07	1 vial
Wash Buffer - 50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution - 11 ml/vial of TMB substrate solution	TMB01	1 vial
Stop Solution - 11 ml /vial of 0.5N HCl	S-STOP	1 vial
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard , Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8°C.

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.

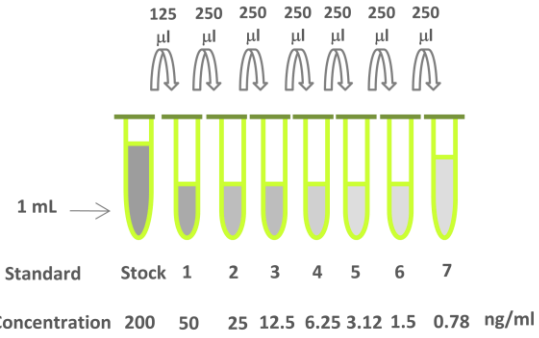
SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20°C or -70°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 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C or -70°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C or -70°C. Avoid repeated freeze-thaw cycles.
Notice: Heparin cannot be used as anticogulant for CHEMERIN ELISA assay.

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 ml of Dilution Buffer. This reconstitution produces a stock solution of 200 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 375 µL of Dilution Buffer into tube #1 and 250 µL into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	Powder	1 ml	200 ng/ml
# 1	125µl of stock	375µl	50 ng/ml
# 2	250µl of 1	250µl	25 ng/ml
# 3	250µl of 2	250µl	12.5 ng/ml
# 4	250µl of 3	250µl	6.25 ng/ml
# 5	250µl of 4	250µl	3.125 ng/ml
# 6	250µl of 5	250µl	1.56 ng/ml
#7	250µl of 6	250µl	0.782 ng/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 120 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 11.88 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB06C)** into a 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution.

Positive Control – Reconstitute Positive Control with 1.0 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Suggestion: Pipette 225 µl of Dilution Buffer into a microcentrifuge tube and transfer 25 µl of 10-fold concentrate stock solution to prepare positive control working solution.

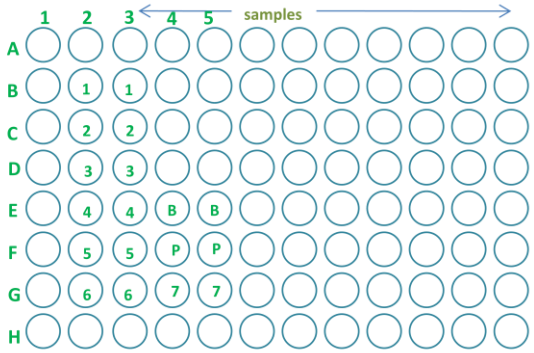
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well (E4, E5).
4. Add 100 µL of **Standard** (B2, B3 to G2, G3 and G4, G5), **sample**, or **positive control** (F4, F5) per well. Cover with plate sealer. Incubate for 2 hours on micro-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 **Wash Buffer** (300 µL) 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 µL of **Detection Antibody working solution** to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 45-60 mins on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 3-10 minutes at room temperature. **Protect from light.**
11. Add 100 µL 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 micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 four parameter logistic (4-PL) 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.



CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human CHEMERIN.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CHEMERIN was 50 pg/ml.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	CORRECTED 450 READING
Blank	0.106
50	1.840
25	1.459
12.5	1.101
6.25	0.745
3.125	0.406
1.563	0.200
0.782	0.078

*Lot No.:

** Positive Control: 1.0 – 2.05 ng/mL

SPECIFICITY

This assay recognizes both natural and recombinant human CHEMERIN. The factors listed below were prepared at 1000 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 1000 ng/mL in a mid-range rh CHEMERIN control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSSREACTIVITY (%)
Human chemerin	100
Mouse chemerin	0
Human FGF-21	0
Human NGAL	0
Human vaspin	0

LINEARITY

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

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
40X	6.977	279.08	100
80X	3.320	265.6	95.2

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

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
40X	10.302	412.08	100
80X	5.282	422.56	102.5

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of **standard, samples, positive control** to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL **Detection Antibody working solution** to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL **Streptavidin HRP conjugate working solution** to each well. Incubate 45-60 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Substrate Solution** to each well. Incubate 3-10 min on the bench top. **Protect from light.**
↓
Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min