

HIGH SENSITIVITY CHEMERIN (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN CHEMERIN CONCENTRATIONS IN SERUM
AND PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HIGH SENSITIVITY CHEMERIN (HUMAN) ELISA KIT
Catalog No.	SK00171-02
Lot No.	
Formulation	96 T
Standard range	15.6-500 pg/mL
Sensitivity	7 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma
Dilution Factor	<i>Optimal dilutions should be determined by each laboratory for each application</i>
Specificity	Human chemerin
Calibration	Human chemerin recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C for 1 month, see page 2-3 for more information
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This High Sensitivity Human Chemerin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Chemerin from serum samples and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Chemerin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human Chemerin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human chemerin. The capture antibody can bind to the human chemerin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human chemerin is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human chemerin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

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

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix 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.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Chemerin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human Chemerin.	401-01-01	1 plate
Human Chemerin Standard – refer to lot of recombinant human Chemerin in a buffered protein base with preservative; lyophilized.	171-02-02	1 vial
Detection Antibody Concentrate – 1.05 ml/vial, 10-fold concentrated of biotinylated antibody solution against human Chemerin with preservative	171-02-03	1 vial
Positive Control –one vial, of human Chemerin recombinant with preservative	171-02-04	1 vial
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	DB01	2 bottles
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB08B	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution - 11 mL of 0.5M HCl solution.	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 1 month. For longer storage up to 10 months, unopened Standard, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be

stored only at 2-8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE PREPARATION

Serum or plasma samples may require at least a 200-fold or 400-fold dilution. A 20-fold dilution is 10 µL sample + 190 µL of Dilution Buffer. Final to make a 200-fold dilution is 30 µL of 20-fold diluted sample solution + 270 µL of Dilution Buffer. Finally, to make a 400-fold dilution is 15 µL of 20-fold diluted sample solution + 285 µL of Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application with a sample 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 1x Wash Buffer.

Chemerin Standard - Reconstitute the Chemerin standard with refer to lot of Dilution Buffer. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Dilution Buffer into the tube #1. Pipette 250 µL of Dilution Buffer into the tube #2 to #6. Use the stock solution to produce a dilution

series (below). Mix each tube thoroughly before the next transfer. The **500 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	Refer to lot	2000 pg/ml
optional	125 µl of stock	125 µl	1000 pg/ml
# 1	150µl of stock	450µl	500 pg/ml
# 2	250µl of 2	250µl	250 pg/ml
# 3	250µl of 3	250µl	125 pg/ml
# 4	250µl of 4	250µl	62.5 pg/ml
# 5	250µl of 5	250µl	31.25 pg/ml
# 6	250µl of 6	250µl	15.6 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to prepare 10-fold concentrated stock. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution (**protect from light**).

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.

4. 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 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit or a 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Chemerin	100
Mouse Chemerin	0
Human Visfatin	0
Human Resistin	0
Human Adiponectin	0

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (refer to lot)
15.6	0.032
31.25	0.068
62.5	0.155
125	0.387
250	0.945
500	2.252
1000 (optional)	3.341

SUMMARY OF ASSAY PROCEDURE

