Quantikine® ELISA

Human Chemerin Immunoassay

Catalog Number DCHM00

For the quantitative determination of human Chemerin concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Chemerin, also known as TIG2 (tazarotene-induced gene 2) and RARRES2 (retinoic acid receptor responder 2), is a distant member of the cystatin/cathelicidin superfamily of proteins (1-3). Human Chemerin is synthesized as a 163 amino acid (aa) precursor with a 20 aa N-terminal signal sequence and a variable length C-terminal pro-segment (3-5). Mature human Chemerin shares 68% aa sequence identity with mouse Chemerin. Serine proteases including plasmin, cathepsins G, K and L, elastase, and tryptase, plus Carboxypeptidases N and B, cleave alone or sequentially to produce maximally active Chemerin (aa 20-157) or partially activated forms (5-11). Alternatively, neutrophil PR3 and mast cell chymase can convert pro and active forms of Chemerin, respectively, to permanently inactive forms (12). Retention of the pro-segment blocks activity, while a peptide representing amino acids preceding the pro-segment (aa 147-155) retains all activity (4, 5). Cells producing Chemerin potentially include adipocytes, endothelial cells, fibroblasts, hepatocytes, keratinocytes, and some cancers such as glioblastoma (2, 5, 13-16). It is present in serum, plasma, cerebrospinal fluid, synovial fluid, and ascitic fluid, and probably occurs in monomeric, dimeric and higher order multimeric forms (3, 7, 13). Chemerin in plasma is not normally activated, but a higher proportion is active in serum due to plasmin cleavage (5, 6).

Activated Chemerin binds to the G-protein coupled receptor ChemR23, also called CMKLR1 (chemokine-like receptor 1), expressed on endothelial cells, mature adipocytes, macrophages, microglia, natural killer (NK) cells, and plasmacytoid and some myeloid-derived dendritic cells (3, 4, 15-20). ChemR23 is implicated in the severity of experimental autoimmune encephalomyelitis, as mice deleted for ChemR23 show less severe disease (18). A second receptor, CCRL2 (chemokine C-C receptor-like 2), binds Chemerin with high affinity, but does not appear to mediate signaling (20, 21). It is thought to act as a non-internalizing interreceptor, concentrating Chemerin on the cell surface for presentation to ChemR23 (9, 20-22). CCRL2 is expressed on many of the same cell types that also express Chemerin and ChemR23, and can be upregulated by lipopolysaccharides or TNF-α on these cells and endothelial cells (9, 20, 22). Another potential receptor, GPR1 (G-protein-coupled receptor 1), can bind Chemerin *in vitro* (23).

Like many other chemokine family members, Chemerin mediates chemoattraction and specifically recruits antigen presenting cells and NK cells that express ChemR23 (3, 4, 6, 14, 16, 20). It is posited that high concentrations of tissue pro-Chemerin become activated by serine proteases released by early-responding inflammatory cells. Both elastase and cathepsin G are components of neutrophil granules, and their release in response to microbial insult can activate Chemerin and induce macrophage/monocyte infiltration (9, 10). Cathepsin K or L cleavage is thought to mediate both chemoattraction and direct antibacterial activity in inflamed lung, joints or skin (11). Chemerin is angiogenic, acting on ChemR23-expressing endothelial cells (20). It has also been shown to activate macrophage adhesion to fibronectin and VCAM-1 by promoting clustering of integrins α4β1 and α5β1 (22, 24). In patients with arthritis, synovial fluid Chemerin may be elevated (7). Differentiating and mature white adipocytes secrete, activate, and respond to Chemerin, promoting their differentiation and function (13-15, 20, 25). Activation of the adipocyte Chemerin receptor increases lipolysis and the efficiency of insulin-induced glucose uptake, thus materially impacting fat metabolism (13-15, 25).

The Quantikine® Human Chemerin Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human Chemerin in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human Chemerin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Chemerin showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this 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 human 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, an enzyme-linked polyclonal antibody specific for human Chemerin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, 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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Chemerin Microplate	893435	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Chemerin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Chemerin Conjugate	893436	21 mL of a polyclonal antibody specific for human Chemerin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Chemerin Standard	893437	Recombinant human Chemerin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	2 vials (21 mL/vial) of a buffered protein base with preservatives. *Use undiluted in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Chemerin Controls (optional; R&D Systems®, Catalog # QC62).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin 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. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require at least a 100-fold dilution. A suggested 100-fold dilution can be achieved by adding 50 µL of sample to 200 µL of Calibrator Diluent RD5-26 Concentrate (undiluted). Complete the 100-fold dilution by adding 20 µL of the diluted sample to 380 µL Calibrator Diluent RD5-26 Concentrate (undiluted).

REAGENT PREPARATION

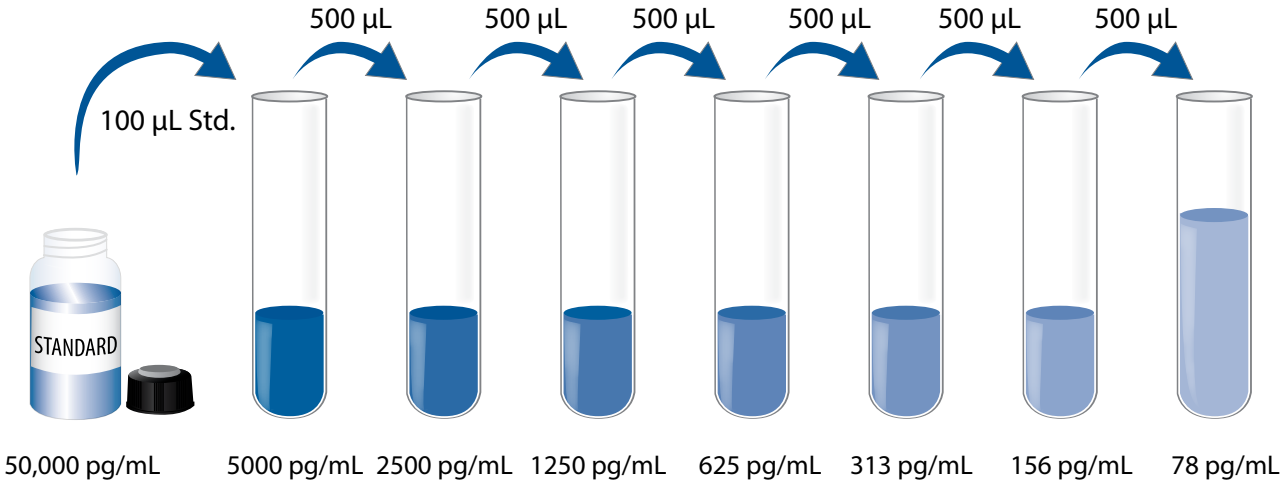
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human Chemerin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Chemerin Standard with deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-26 Concentrate (undiluted) into the 5000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-26 Concentrate (undiluted) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 (400 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 200 µL of Human Chemerin Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

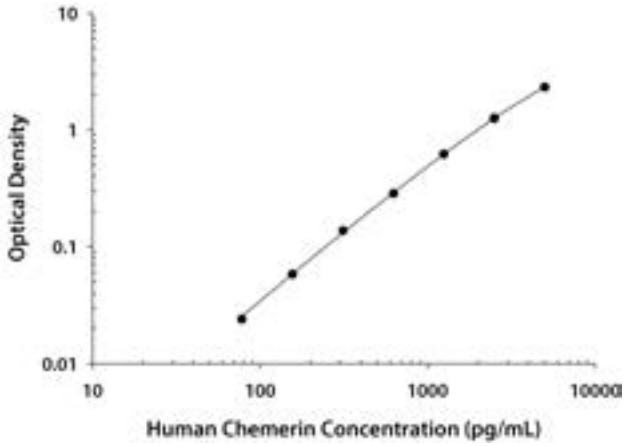
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human 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.

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.008 0.010	0.009	—
78	0.031 0.034	0.033	0.024
156	0.065 0.068	0.067	0.058
313	0.144 0.147	0.146	0.137
625	0.292 0.295	0.294	0.285
1250	0.625 0.635	0.630	0.621
2500	1.253 1.276	1.265	1.256
5000	2.305 2.340	2.323	2.314

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	513	1558	3016	515	1592	3147
Standard deviation	23.3	66.5	84.6	40.5	121	200
CV (%)	4.5	4.3	2.8	7.9	7.6	6.4

RECOVERY

The recovery of human Chemerin spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	104	94-111%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Chemerin were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=8)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	87	96	96	98
	Range (%)	84-91	95-97	95-97	94-106
1:4	Average % of Expected	85	94	90	97
	Range (%)	81-91	90-96	85-94	89-105
1:8	Average % of Expected	86	95	89	96
	Range (%)	81-94	93-97	87-91	85-108
1:16	Average % of Expected	88	93	90	95
	Range (%)	83-98	86-101	80-97	90-103

*Samples were diluted prior to assay.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human Chemerin ranged from 1.08-7.80 pg/mL. The mean MDD was 4.13 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human Chemerin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Chemerin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	83.5	48.0-142	22.1
EDTA plasma (n=35)	71.9	34.5-146	24.7
Heparin plasma (n=35)	96.0	60.1-199	31.9

Cell Culture Supernates:
OVCAR-3 human ovarian carcinoma cells were cultured in RPMI and supplemented with 20% fetal bovine serum, 10 µg/mL bovine insulin, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Chemerin, and measured 0.161 ng/mL.

HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Chemerin, and measured 31.3 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Chemerin.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Chemerin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Adiponectin
Cystatin A
Cystatin B
Cystatin C
Cystatin D
Cystatin E/M
Cystatin F
Cystatin S
Fetuin A
Fetuin B
Leptin
Resistin

Recombinant mouse:

Chemerin

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