Human CX3CL1 Immunoassay

Catalog Number: EA800057

For the quantitative determination of human CX3CL1 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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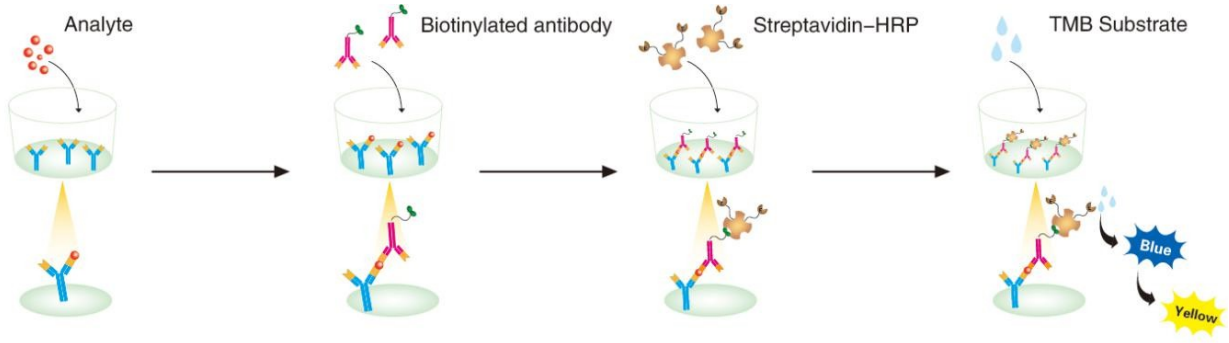
BACKGROUND

Fractalkine, also known as CX3CL1 or neurotactin, is the only member of the CX3C or delta chemokine subfamily. Fractalkine is produced as a transmembrane protein, unlike all other chemokines except CXCL16. The 95-100 kDa type I transmembrane form of Fractalkine can be proteolytically processed to generate a secreted 60-80 kDa soluble form. Soluble Fractalkine has been identified in serum, urine, cerebrospinal, amniotic and synovial fluids. Human Fractalkine shares 60% or 63% aa sequence identity within the entire extracellular domain, and 78% or 85% aa sequence identity within the chemokine domain only, with mouse or rat Fractalkine, respectively. Fractalkine is predominantly expressed by vascular endothelium and smooth muscle, neurons, dendritic cells, and the epithelial linings of the intestine, bronchi, renal proximal tubules, endometrium, fallopian tube, and bile duct. Most Fractalkine expression is induced by inflammatory cytokines such as TNF-α and IFN-γ, but expression on forebrain neurons as well as Leydig and Sertoli testicular cells is also constitutive. Expression by astrocytes, osteoblasts, stratum basal keratinocytes, and fibroblasts has also been reported.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CX3CL1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CX3CL1 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for CX3CL1 is added to detect the captured CX3CL1 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



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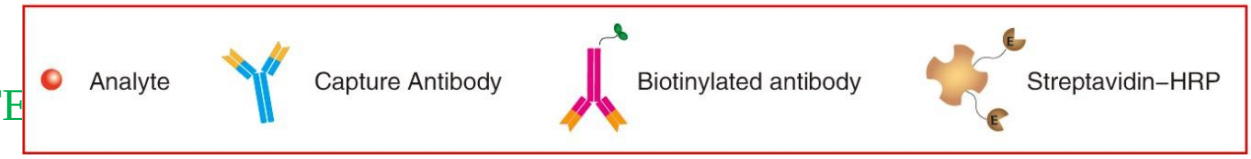


1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	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℃**
Standard - lyophilized, 20000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard/Sample Diluent - 16 ml/ vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

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

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

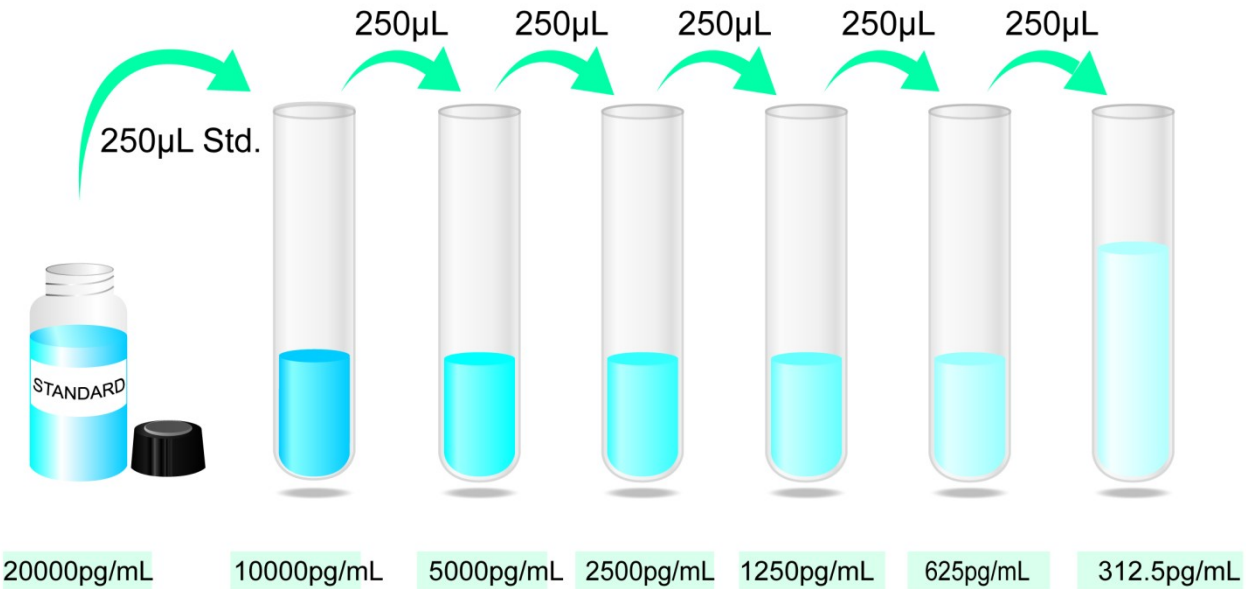
Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. 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 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Specimen** - Reconstitute the Standard with 0.5mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 20000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Standard/Sample Diluent into 10000pg/ml tube and the remaining tubes. Use the stock solution of 20000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 20000 pg/mL standard serves as the high



Preparation of CX3CL1 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human CX3CL1 antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human CX3CL1 antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 15 minutes,37℃.Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

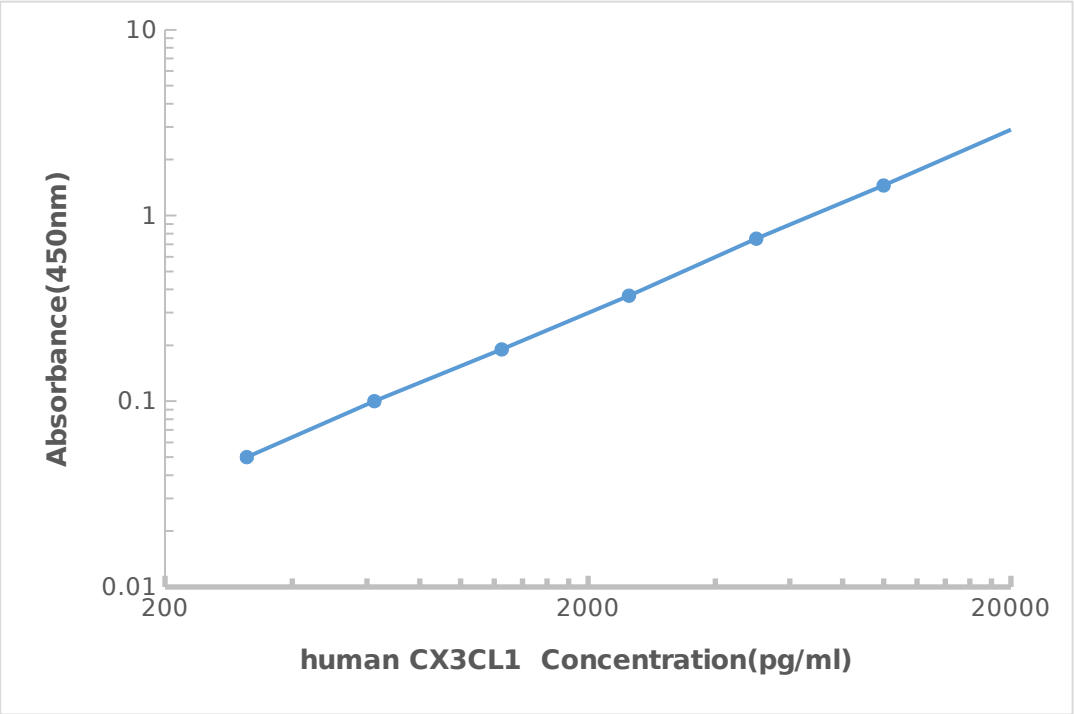
CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the CX3CL1 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.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the CX3CL1 ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	0.052	0.056	0.054	---
312	0.076	0.080	0.078	0.024
625	0.175	0.182	0.178	0.124

1250	0.284	0.296	0.290	0.236
2500	0.425	0.445	0.435	0.381
5000	0.783	0.757	0.770	0.716
10000	1.369	1.386	1.377	1.323
20000	2.468	2.442	2.455	2.401



Representative standard curve for CX3CL1 ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 150pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human CX3CL1. The factors

listed below were prepared at 100ng/ml in Standard /Sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
CX3CR1	CXCL6/GCP-2/LIX	
CXCL6/GCP-2/LIX		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of CX3CL1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of CX3CL1 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	95	88-107
Cell culture supernatants	104	97-116

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of CX3CL1 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	95	104
	Range (%)	86-106	95-116
1:4	Average% of Expected	97	105
	Range (%)	95-114	102-114
1:8	Average% of Expected	98	103
	Range (%)	90-105	92-110
1:16	Average% of Expected	101	105
	Range (%)	93-110	95-114

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