Cx3cl1 (Mouse) ELISA Kit

Catalog Number KA0663

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

For the quantitation of Mouse CX3CL1 concentrations in cell culture supernates, serum and plasma (heparin, EDTA).

Background

Chemokine (C-X3-C motif) ligand 1 (CX3CL1) is a large cytokine protein of 373 amino acids, it contains multiple domains and is the only known member of the CX3C chemokine family. It is also commonly known under the names fractalkine (in humans) and neurotactin (in mice). CX3CL1 is produced as a long protein (with 373-amino acid in humans) with an extended mucin-like stalk and a chemokine domain on top. The mucin-like stalk permits it to bind to the surface of certain cells. However a soluble (90 kD) version of this chemokine has also been observed. Soluble CX3CL1 potently chemoattracts T cells and monocytes, while the cell-bound chemokine promotes strong adhesion of leukocytes to activated endothelial cells, where it is primarily expressed. CX3CL1 elicits its adhesive and migratory functions by interacting with the chemokine receptor CX3CR1. Its gene is located on human chromosome 16 along with some CC chemokines known as CCL17 and CCL22. It can act as a mediator of smooth muscle cell migration in human atherosclerosis, rather than mediate inflammatory cell recruitment.

Principle of the Assay

The Cx3cl1 (Mouse) ELISA Kit is a solid phase immunoassay specially designed to measure Mouse CX3CL1 with a 96-well strip plate that is pre-coated with antibody specific for CX3CL1. The detection antibody is a biotinylated antibody specific for CX3CL1. The capture antibody is a monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Mouse CX3CL1 with immunogen: Expression system for standard: sf21; Immunogen sequence: Q25-R337. The kit is analytically validated with ready to use reagents.

To measure Mouse CX3CL1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Mouse CX3CL1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse CX3CL1 in the sample.

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General Information

Materials Supplied

List of component

Component	Amount
Anti-Mouse CX3CL1 Pre-coated 96-well strip microplate	96 (8x12) wells
Mouse CX3CL1 standard	20 ng/tube x 2
Mouse CX3CL1 Biotinylated antibody (100x)	130 µL
Avidin-Biotin-Peroxidase Complex (100x)	130 µL
Sample Diluent	30 mL
Antibody Diluent	12 mL
Avidin-Biotin-Peroxidase Diluent	12 mL
Color Developing Reagent (TMB)	10 mL
Wash Buffer Powder	1 pack
Stop solution	10 mL
Plate Sealers	4 slides

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

✓   Microplate Reader capable of reading absorbance at 450 nm.

✓   Automated plate washer (optional)

✓   Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

✓   Multichannel pipettes are recommended for large amount of samples.

✓   Deionized or distilled water.

✓   500 mL graduated cylinders.

✓   Test tubes for dilution.

Precautions for Use

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

✓ Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.

2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.

3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.

4. Don't reuse tips and tubes to avoid cross contamination.

5. Avoid using the reagents from different batches together.

Assay Protocol

Reagent Preparation

- ✓ Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (20-25 min) is based on 37°C.

  - Wash buffer – Dissolve the included powder in 1000 mL of deionized water. Excess water buffer can be stored for up to one week at 4°C.

  - Biotinylated Anti-Mouse CX3CL1 antibody - It is recommended to prepare this reagent immediately prior to use by diluting the Mouse CX3CL1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 μL by adding 1 μL of Biotinylated antibody (100x) to 99 μL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

  - Avidin-Biotin-Peroxidase Complex - It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 μL by adding 1 μL of Avidin-Biotin-Peroxidase Complex (100x) to 99 μL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

  - Mouse CX3CL1 Standard - It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 20 ng of lyophilized Mouse CX3CL1 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 20 ng/mL using 1 mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

  - Microplate - The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

- ✓ Dilution of Mouse CX3CL1 Standard

  1. Number tubes 1-8. Final Concentrations to be Tube # 1 –20000 pg/mL, #2 –10000 pg/mL, #3 – 5000 pg/mL, #4 – 2500 pg/mL, #5 – 1250 pg/mL, #6 –625 pg/mL, #7 – 312.5 pg/mL, #8 – 0.0 (Blank).

  2. To generate standard #1, add 1000 μL of undiluted standard stock solution to tube #1.

  3. Add 300 μL of sample diluent to tubes # 2-7.

  4. To generate standard #2, add 300 μL of standard #1 from tube #1 to tube #2 for a final volume of 600 μL. Mix thoroughly.

  5. To generate standard #3, add 300 μL of standard #2 from tube #2 to tube #3 for a final volume of 600 μL. Mix thoroughly.

  6. Continue the serial dilution for tube #4-7.

  7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation

✓ Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

- Cell culture supernates: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.

- Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

- Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1000 x g. Assay immediately or store sample at -20°C.
  Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

✓ Sample Dilution Guideline

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µL of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Reagent Preparation if you have missed this information).

1. Prepare all reagents and working standards as directed previously.

2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.

3. Add 100 µL of the standard, samples, or control per well. Add 100 µL of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.

4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 minutes at 37°C).

5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

6. Add 100 µL of the prepared 1x Biotinylated Anti-Mouse CX3CL1 antibody to each well.

7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).

8. Wash the plate 3 times with the 1x wash buffer.

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the

benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 2 additional times.

9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT or 30 minutes at 37°C.

10. Wash the plate 5 times with the 1x wash buffer.

a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 4 additional times.

11. Add 90 µL of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.).

12. Add 100 µL of Stop Solution to each well. The color should immediately change to yellow.

13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm.

Data Analysis

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

✓ Cx3cl1 (Mouse) ELISA Kit Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/mL)	0	312	625	1250	2500	5000	10000	20000
O.D	0.094	0.110	0.156	0.253	0.566	0.924	1.437	1.982
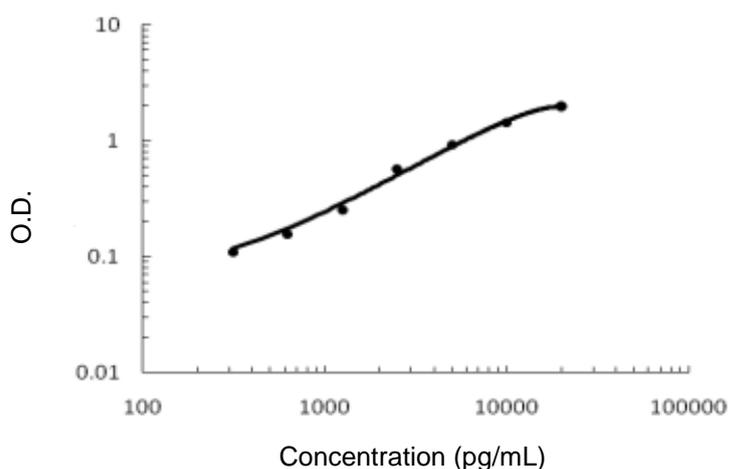
A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Performance Characteristics

✓ Detection Range: 312 pg/mL- 20000 pg/mL

✓ Sensitivity: < 10 pg/mL

*The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

✓ Specificity: Natural and recombinant Mouse CX3CL1.

✓ Cross-reactivity: There is no detectable cross-reactivity with Eotaxin, MCP-1, MCP-2, 6Ckine, MARC.

✓ Intra/Inter Assay Variability

- Intra-Assay Precision (Precision within an assay)

  Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision (Precision across assays)

  Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	378	2168	9077	392	1975	9206
Standard deviation	17.76	101.89	653.54	23.52	114.55	810.12
CV (%)	4.7%	4.7%	7.2%	6%	5.8%	8.8%

✓ Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/mL)	Lot2 (pg/mL)	Lot3 (pg/mL)	Lot4 (pg/mL)	Mean (pg/mL)	Standard Deviation	CV (%)
Sample 1	378	415	410	441	411	22.39	5.4%
Sample 2	2168	2082	1886	2198	2083	121.71	5.8%
Sample 3	9077	9811	9555	9593	9509	267.86	2.8%

*number of samples for each test n=16.

Resource

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												