Human CX3CL1 ELISA Kit

Enzyme Immunoassay for the quantification of CX3CL1 in human serum, plasma and cell culture supernatants samples

Catalog number: ARG81375

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

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INTRODUCTION

Fractalkine also known as chemokine (C-X3-C motif) ligand 1 is a protein that in humans is encoded by the CX3CL1 gene. CX3CL1 gene belongs to the CX3C subgroup of chemokines, characterized by the number of amino acids located between the conserved cysteine residues. This is the only member of the CX3C subgroup, which contains three amino acids between cysteine residues, resulting in a Cys-X-X-X-Cys configuration. The encoded protein contains an extended mucin-like stalk with a chemokine domain on top, and exists in both a membrane-anchored form where it acts as a binding molecule, or, in soluble form, as a chemotactic cytokine. The mature form of this protein can be cleaved at the cell surface, yielding different soluble forms that can interact with the G-protein coupled receptor, C-X3-C motif chemokine receptor 1 gene product. This gene plays a role in a wide range of diseases, including cancer, vasculitis, neuropathies, atherosclerosis, inflammatory diseases, and in human immunodeficiency virus infections. [provided by RefSeq, Sep 2017]

Fractalkine protein acts as a ligand for both CX3CR1 and integrins. Binds to CX3CR1 (PubMed:23125415, PubMed:9931005, PubMed:21829356). Binds to integrins ITGAV:ITGB3 and ITGA4:ITGB1. Can activate integrins in both a CX3CR1-dependent and CX3CR1-independent manner. In the presence of CX3CR1, activates integrins by binding to the classical ligand-binding site (site 1) in integrins. In the absence of CX3CR1, binds to a second site (site 2) in integrins which is distinct from site 1 and enhances the binding of other integrin ligands to site 1 (PubMed:23125415, PubMed:24789099). The soluble form is chemotactic for T-cells and monocytes and not for neutrophils. The membrane-bound form promotes adhesion of those leukocytes to endothelial cells. May play a role in regulating leukocyte adhesion and migration processes at the endothelium (PubMed:9024663, PubMed:9177350). [provided by

uniprot]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CX3CL1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CX3CL1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for CX3CL1 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CX3CL1 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of CX3CL1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody coated microplate	12 x 8 wells	4°C
20X Wash Buffer	50 ml	4°C
Biotin-conjugated antibody	1 vial	4°C
Standard	1 vial	4°C
2000X Streptavidin-HRP conjugate	30 µl	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution (2N HCl)	15 ml (Ready-to-use)	4°C
Positive Controls (Accept. Range dependent on lot#)	2 vials	4°C
Plate sealer	3 pieces	Room Temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (300-400rpm)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Before opening any Eppendorf tubes for reconstitution, briefly centrifuge at ~3,000rpm for 5 seconds to ensure that all the lyophilized material is at the bottom of the tube.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that all solutions be used as soon as possible after reconstitution.
- Unused microplate strips should be placed back in the foil pouch with a desiccant and stored at 4°C. Do not allow moisture to enter the wells.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.
- For optimal results, an orbital plate shaker capable of 300-400 rpm is recommended for all incubations.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & 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 before centrifugation for 15 minutes at 1000 x g. Collect 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:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 20X Wash Buffer into distilled water to yield 1X Wash buffer. Store the diluted 1X Wash Buffer at 4°C. If crystals appear in 20X Wash Buffer, warm the buffer in warm water bath (not higher than 50°C) for 30 minutes or until crystals disappear. Mix well before use.
- **Biotin-conjugated antibody**: Reconstitute Biotin-conjugated antibody with 100 μl of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, mix well and centrifuge the tube before use. Keep rehydrated solution at 4°C. Store the reconstituted antibody at 4°C up to a week. For long-term storage, aliquot & store at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles. It is recommended that antibody should only be frozen-thawed once. Dilute Biotinylated conjugated antibody to 1:250 with 1X Wash Buffer and mix thoroughly before use. (E.g. Add 50 μl of Biotin-conjugated antibody into 12450 μl of 1X Wash Buffer, mix well)
- **Positive control**: Centrifuge and reconstitute the Positive control vial with 250 μl of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, vortex to mix well and centrifuge the tube before use. Keep rehydrated solution at 4°C before use. Store the reconstituted control at 4°C up to a week. For long-term storage, aliquot & store at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles. It is recommended that control should only be frozen-thawed once. (acceptable range dependent on lot#)

- **1X Streptavidin-HRP conjugate:** 1X Streptavidin-HRP conjugate working solution should be prepared freshly before use. Centrifuge 2000X Streptavidin-HRP conjugate briefly (3,000-5,000 rpm, 5 seconds) and add 6 µl of Streptavidin-HRP to 12 ml 1X wash buffer to make a 1X Streptavidin-HRP working solution. Vortex thoroughly.
- **Standard**: Centrifuge and reconstitute the standard with 1 ml of 1X Wash buffer and vortex. The concentration of this stock solution is 100 ng/ml. Allow the solution to sit for at least 10 minutes at room temperature to completely dissolve. Store the reconstituted standard at 4°C up to a week. For long-term storage, aliquot & store at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles. It is recommended that standard should only be frozen-thawed once. Dilute standard solutions with 1X Wash buffer to 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml and 0.3125 ng/ml as follows:

The example of the dilution of standards

Standard No.	Standard Conc. (ng/ml)	1X Wash Buffer (µl)	Standard (µl)
Stock	100	-	-
S1	20	800	200 µl of Stock
S2	10	500	500 µl of S1
S3	5	500	500 µl of S2
S4	2.5	500	500 µl of S3
S5	1.25	500	500 µl of S4
S6	0.625	500	500 µl of S5
S7	0.3125	500	500 µl of S6
S0 (Total binding)	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-23°C) 30 minutes before use. Standards, samples and blank should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Keep the foil pouch at 4°C.
2. Aspirate each well and wash each well once with **1× Wash Buffer (350 µl)** using a squirt bottle, manifold dispenser, or autowasher. Keep the buffer in the wells for at least 5 min. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Do not let wells dry before proceeding to the next step.
3. Add **100 µl** of **standards, controls, samples and zero controls** (1X wash buffer) into wells, gently tap the plate to mix well.
4. Seal the microtiter plate with plate sealer. Incubate for **2 hours at RT (20-23°C).** Orbital shaking at 300-400 rpm is recommended.
5. Remove sealer from plate carefully.
6. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes**. Wash by filling each well with **1× Wash Buffer (350 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **100 µl of 1X Biotin-conjugated antibody** into each well.

8. Seal the microtiter plate with plate sealer. Incubate for **2 hours at RT (20-23°C)** on an orbital microplate shaker at 300-400 rpm.
9. Remove sealer from plate carefully. **Wash** as according to step 6
10. Add **100 µl of diluted (1X) Streptavidin-HRP** working solution into **each well**.
11. Incubate for **30 min at RT** on an orbital microplate shaker at 300-400 rpm.
12. **Wash** as according to step 6.
13. Add **100 µl of TMB substrate** solution into **each well**.
14. Incubate for **20-30 min at RT in dark** on an orbital microplate shaker at 300-400 rpm.
15. Add **100 µl of STOP solution (2N HCl)** into **each wells** to stop the reaction. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
16. Read the OD with a microplate reader at 450 nm immediately. It is recommended that the wells be read within 20 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

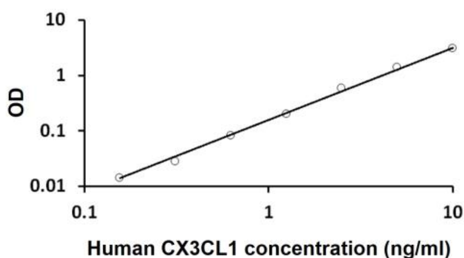
4. Automated method: The results in the IFU have been calculated

automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The standard of CX3CL1 ranged from 0-20 ng/ml.

The mean MDD was 0.156 ng/ml.

Linear Range

0.156 – 10 ng/ml

Precision:

Intra-assay: < 10%

Inter-assay: < 15%