

# Human CX3CL1 ELISA Kit

## Sandwich ELISA

### Catalog EL-213-32403

#### INTRODUCTION

CX3CL1, commonly called fractalkine, is the sole human chemokine of the CX3C class and is unusual in existing both as a membrane-tethered molecule with a mucin-like stalk and, after proteolytic shedding, as a soluble mediator. Endothelial cells display the membrane-bound form following exposure to inflammatory signals such as interferon-gamma and tumor necrosis factor-alpha, allowing it to grab and firmly anchor circulating monocytes, T cells, and NK cells at the vessel wall. Once cleaved free, the soluble fragment instead acts as a classic chemoattractant, drawing monocytes and T lymphocytes toward sites of injury or inflammation through its single receptor, CX3CR1, which is concentrated on cytotoxic lymphocytes and macrophages. This chemokine-receptor pairing has been implicated in a wide array of conditions, including atherosclerosis, vasculitis, several cancers, and chronic inflammatory and neurological disorders.

#### PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format to detect Human CX3CL1. The microtiter plate wells are pre-coated with a CX3CL1-specific capture antibody, which binds the target protein present in standards or samples. A biotin-conjugated detection antibody, also specific to Human CX3CL1, is then added and binds a second epitope on the captured analyte. Avidin conjugated to horseradish peroxidase (HRP) is subsequently introduced and binds the biotin label. Upon addition of TMB substrate solution, a colorimetric signal develops only in wells where CX3CL1, the biotin-conjugated detection antibody, and the avidin-HRP conjugate are all present. The enzymatic reaction is stopped by the addition of sulphuric acid solution, and absorbance is measured at 450 nm ( $\pm$  10 nm). CX3CL1 concentrations in unknown samples are determined by comparing their optical density values against a standard curve.

#### MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

| Each kit includes:                                                   | Units               |
|----------------------------------------------------------------------|---------------------|
| Pre-Coated Microplate                                                | 12 strips x 8 wells |
| Standard (Lyophilized)                                               | 2 vials             |
| Biotinylated Antibody (100×)                                         | 120 µL              |
| Streptavidin-HRP (100×)                                              | 120 µL              |
| Standard/Sample Diluent Buffer                                       | 20 mL               |
| Biotinylated Antibody Diluent                                        | 12 mL               |
| HRP Diluent                                                          | 12 mL               |
| Wash Buffer (25×)                                                    | 20 mL               |
| TMB Substrate Solution                                               | 10 mL               |
| Stop Reagent                                                         | 6 mL                |
| Plate Covers                                                         | 2 pieces            |
| <i>Do not mix or substitute reagents with those from other lots.</i> |                     |

#### MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

#### SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective

clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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#### **PREPARATION OF REAGENTS**

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 10 down to 0.16 (plus a 0 blank), per the source manual.**

| Sol'n ID | Source         | Source Vol (µL) | Cal. Diluent (µL) | Final Vol (µL) | Final Conc. (ng/mL) |
|----------|----------------|-----------------|-------------------|----------------|---------------------|
| Std 1    | Stock Standard | 500             | 500               | 1000           | 10                  |
| Std 2    | Std 1          | 500             | 500               | 1000           | 5                   |
| Std 3    | Std 2          | 500             | 500               | 1000           | 2.5                 |
| Std 4    | Std 3          | 500             | 500               | 1000           | 1.25                |
| Std 5    | Std 4          | 500             | 500               | 1000           | 0.63                |
| Std 6    | Std 5          | 500             | 500               | 1000           | 0.32                |
| Std 7    | Std 6          | 500             | 500               | 1000           | 0.16                |
| Blank    | Std 7          | 500             | 500               | 1000           | 0                   |

#### **SPECIMEN STORAGE**

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

#### **ASSAY PROCEDURE**

1. After the kit is equilibrated at room temperature, add 100 µL of Standard Working Buffer (gradually diluted according to the instructions) or 100 µL of sample to each well, and incubate at 37°C for 80 minutes.
2. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 µL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.
3. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 µL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.
4. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 µL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.
5. Add 50 µL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

#### **CALCULATIONS AND RESULTS**

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.

3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

#### **PERFORMANCE CHARACTERISTICS**

**Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.**

**Detection Limit: 0.066 ng/mL**

**Recovery: Serum (n = 5): 80-94% (avg 87%); EDTA plasma (n = 5): 91-105% (avg 98%); Heparin plasma (n = 5): 85-97% (avg 91%)**

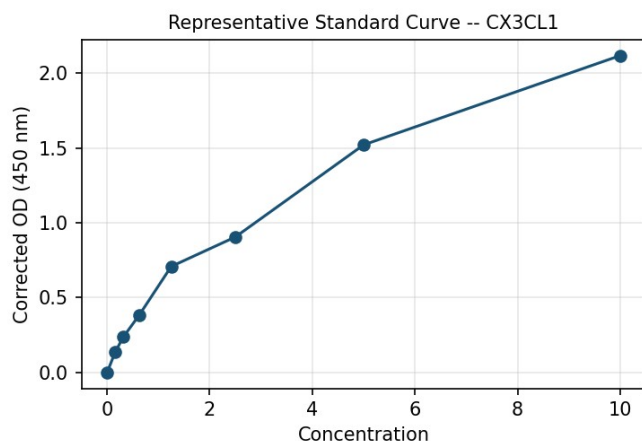
**Specificity: This assay has high sensitivity and excellent specificity for detection of Human CX3CL1. No significant cross-reactivity or interference between Human CX3CL1 and analogues was observed.**



**This kit employs a sandwich enzyme immunoassay technique to quantify Human CX3CL1 in biological samples.**

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### **SAMPLE STANDARD CURVE**



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

### **ORDERING INFORMATION**

Please visit [www.affinityimmuno.com](http://www.affinityimmuno.com) to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.