Quantikine® ELISA

Human CXCL16 Immunoassay

Catalog Number DCX160

For the quantitative determination of human CXCL16 concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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

CXCL16 is a member of the chemokine superfamily with putative roles in directing leukocyte migration and functioning as a scavenger receptor. The gene for human CXCL16 predicts a 254 amino acid (aa) protein with an N-terminal signal peptide of 27 aa (1-3). It is a putative type I transmembrane protein with a non-ELR motif-containing a CXC chemokine domain, a mucin-like spacer region, a transmembrane domain, and a cytoplasmic domain with consensus tyrosine phosphorylation and SH2 binding sites (1, 2). CXCL16 and Fractalkine (CX3CL1) constitute the only transmembrane chemokines described, although some CXCL16 activity likely results from a functional 35 kDa soluble form shed from the membrane (1, 2, 4). Mouse and human CXCL16 share 49% overall aa sequence identity with the greatest degree of similarity found in the chemokine domain (1, 3). CXCL16 has also been independently cloned as SR-PSOX (scavenger receptor that binds phosphatidylserine and oxidized lipoprotein) (3).

CXCL16 expression is detected in various lymphoid tissues including thymus, spleen, Peyer's patches, and lymph nodes, and in non-lymphoid tissue including kidney, lung, small intestine, liver, and atherosclerotic lesions (1, 2, 5). CXCL16 is found primarily on the surface of antigen-presenting cells (APCs) including $CD19^+$ B cells, monocytes/macrophages, and dendritic cells, although it may be expressed by some T cells, smooth muscle cells, and endothelial cells as well (1, 3, 6-8).

The receptor for CXCL16 has been identified as CXCR6 (also known as Bonzo, STRL33 or TYMSTR), a protein previously shown to be a co-receptor for HIV entry (9-12). CXCR6 is expressed on naive $CD8^+$ T cells, Th1 polarized $CD8^+$ and $CD4^+$ T cells, NK T cells, plasma cells, and astrocytes (1, 2, 13-16). CXCR6 is enriched in cells found at sites of inflammation (13).

CXCL16 has the ability to stimulate CXCR6-dependent chemotaxis, and in T cells this activity is pertussis toxin-sensitive (1, 2). In plasma cells, CXCL16 can stimulate chemotactic migration and enhances adhesion to fibronectin (15). Myeloma cells adhere to immobilized CXCL16 in a manner dependent upon CXCR6, but not requiring pertussis toxin-sensitive G-proteins or integrin activity (15). In addition, CXCL16 is shown to act as a scavenger receptor, mediating the uptake of phosphatidylserine and oxidized low density lipoprotein (Ox-LDL), and the phagocytosis by APCs of both gram-negative and gram-positive bacteria (3, 17). The bacterial recognition sequence appears to reside in the chemokine domain (17).

The Quantikine Human CXCL16 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human CXCL16 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human CXCL16 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CXCL16 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Human CXCL16 kit can be used to determine relative mass values for naturally occurring human CXCL16.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human CXCL16 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CXCL16 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human CXCL16 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 CXCL16 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, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard 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 CXCL16 Microplate	892810	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human CXCL16.	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 CXCL16 Conjugate	892811	21 mL of a polyclonal antibody specific for human CXCL16 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human CXCL16 Standard	892812	Recombinant human CXCL16 in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5R	895190	21 mL of a buffered protein base with preservatives.	
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.
- Test tubes for dilution of standards.
- Human CXCL16 Controls (R&D Systems, Catalog # QC72).

PRECAUTIONS

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

Some components in this kit contain ProClin® 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. Please refer to the MSDS 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.*

REAGENT PREPARATION

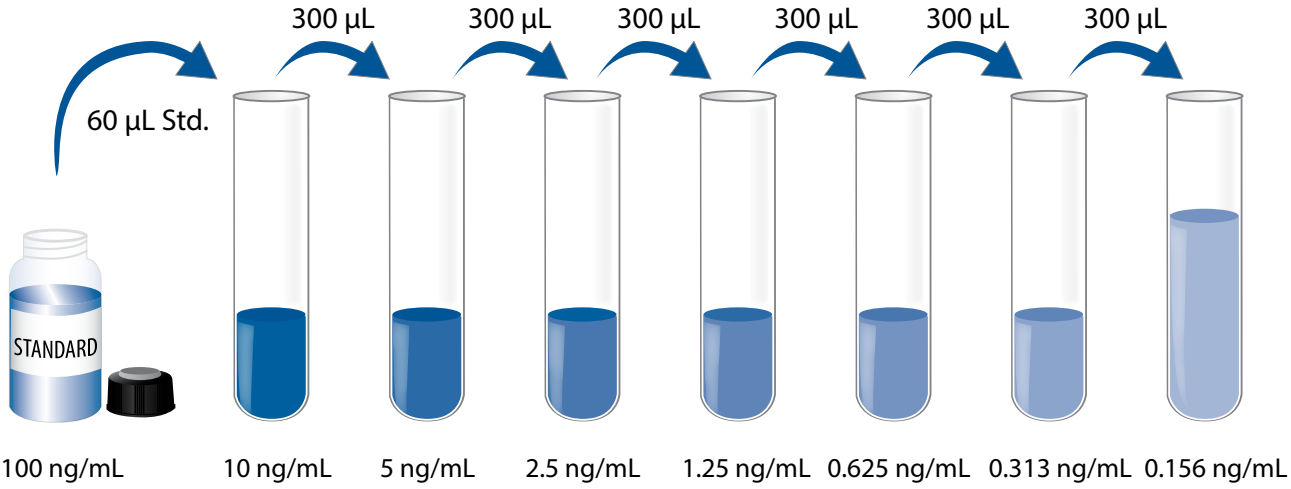
Bring all reagents to room temperature before use.

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 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 CXCL16 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CXCL16 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 540 µL of Calibrator Diluent RD5R into the 10 ng/mL tube. Pipette 300 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5R serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards 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 RD1W 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.
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 CXCL16 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
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. **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.

CALCULATION OF RESULTS

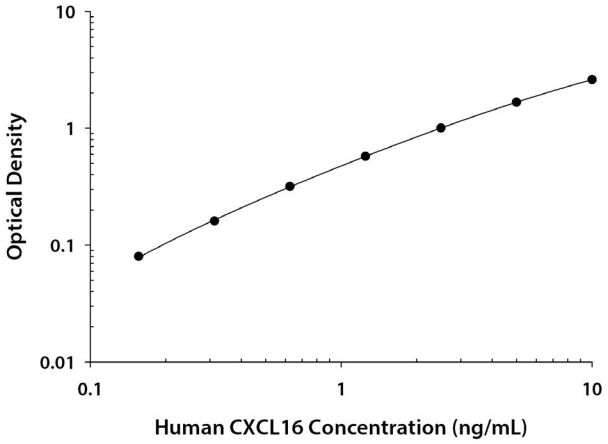
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 CXCL16 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.



(ng/mL)	O.D.	Average	Corrected
0	0.021 0.022	0.022	—
0.156	0.102 0.102	0.102	0.080
0.313	0.181 0.185	0.183	0.161
0.625	0.337 0.340	0.339	0.317
1.25	0.585 0.607	0.596	0.574
2.5	1.022 1.029	1.026	1.004
5	1.679 1.702	1.691	1.669
10	2.614 2.631	2.623	2.601

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 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	40	42	42
Mean (ng/mL)	0.92	2.85	6.33	1.00	3.30	7.30
Standard deviation	0.04	0.10	0.31	0.10	0.30	0.70
CV (%)	4.3	3.5	4.9	10.0	9.1	9.6

RECOVERY

The recovery of human CXCL16 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	93-110%
Serum (n=4)	103	89-113%
EDTA plasma (n=4)	102	94-112%
Heparin plasma (n=4)	97	85-105%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human CXCL16 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	100	103	102	99
	Range (%)	98-101	99-107	98-105	94-102
1:4	Average % of Expected	101	107	105	103
	Range (%)	100-103	104-113	102-110	101-105
1:8	Average % of Expected	99	107	106	106
	Range (%)	98-100	104-113	103-107	102-109
1:16	Average % of Expected	99	103	100	107
	Range (%)	97-101	99-110	96-101	102-114

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human CXCL16 ranged from 0.003-0.017 ng/mL. The mean MDD was 0.007 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density 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 CXCL16 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human CXCL16 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)	2.32	1.01-3.44	0.51
EDTA plasma (n=35)	2.07	0.99-3.92	0.58
Heparin plasma (n=35)	2.13	1.10-3.29	0.48

Cell Culture Supernates:
Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of natural human CXCL16.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	0.34	1.51
Stimulated	0.28	1.57

Additional cell lines were tested for the presence of human CXCL16.

Cell Line	Values (ng/mL)
HepG2	2.65
Monocytes stimulated with GM-CSF	0.45
Monocytes stimulated with IL-4	0.31

SPECIFICITY

This assay recognizes natural and recombinant human CXCL16.

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 CXCL16 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
BLC/BCA-1
ENA-78
Fractalkine
GCP-2
GROα
GROβ
GROγ
IL-8
IL-8 (endothelial cell-derived)
IP-10
I-TAC
MIG
NAP-2
SDF-1α
SDF-1β

Recombinant mouse:
BLC/BCA-1
CRG-2
GCP-2
I-TAC
KC
MIG
SDF-1α

Recombinant porcine:
IL-8

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