Quantikine® ELISA

Human CXCL12/SDF-1α Immunoassay

Catalog Number DSA00
SSA00
PDSA00

For the quantitative determination of human Stromal cell-Derived Factor 1 alpha (SDF-1α) concentrations in cell culture supernates and platelet-poor plasma.

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

SDF-1 is a CXC chemokine (α sub-family) with unusual characteristics (1-5). It has weak sequence homology with other CXC chemokines and with members of the CC (β) chemokine sub-family, appearing to represent an additional sub-family of chemokines (4). In contrast to other chemokine genes, which are on chromosomes 4 or 17, the SDF-1 gene is on chromosome 10 (3). SDF-1 also has a higher degree of inter-species conservation than other chemokines, with 99% identity between the human and the mouse sequences (1, 3).

There are two alternatively spliced variants of SDF-1 (1). SDF-1α is an 89 amino acid polypeptide, while SDF-1β has the identical sequence with a 4-residue C-terminal extension. SDF-1 is a highly basic (1), heparin-binding protein (4). There is no glycosylation site in the sequence of SDF-1. SDF-1 binds to and activates the CXCR4 receptor (LESTR/fusin) (5, 6). The receptor activation involves dimerization, interaction with $G\alpha_i$ (alpha subunit of inhibitory G protein), phosphorylation by JAK2/JAK3 kinase (janus kinase, non-receptor protein tyrosine kinase) and phosphorylation of signal transducers and activators of transcription (STAT) factors (7).

SDF-1 is constitutively expressed by bone marrow stromal cells and is present in many other tissues (1, 2, 4). It is a potent chemoattractant for lymphocytes and monocytes, and it enhances B-cell proliferation (1, 2, 4), leading to the suggestion that it may be an agent of immune surveillance rather than a mediator of the inflammatory response (4). It appears to play a role in trafficking or homing of lymphocytes and hematopoietic cells (8).

The Quantikine® Human CXCL12/SDF-1α Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human SDF-1α in cell culture supernates and platelet-poor plasma. It contains *E. coli*-expressed recombinant human SDF-1α and antibodies raised against the recombinant factor. It has been shown to accurately quantitate recombinant human SDF-1α. Results obtained using natural SDF-1α showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human SDF-1α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human SDF-1α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SDF-1α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human SDF-1α 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 SDF-1α 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, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- 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.

PRECAUTIONS

Calibrator Diluent RD6Q contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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

Some components in this kit contain a preservative 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. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DSA00	CATALOG # SSA00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human SDF-1α Microplate	890813	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human SDF-1α.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human SDF-1α Standard	890815	2 vials	12 vials	Recombinant human SDF-1α in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use within 2 hours of reconstitution. Use a new standard for each assay. Discard after use.
Human SDF-1α Conjugate	890814	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human SDF-1α conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-55	895066	1 vial	6 vials	11 mL/vial of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD6Q	895128	1 vial	6 vials	21 mL/vial of animal serum with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DSA00 contains sufficient materials to run an ELISA on one 96 well plate.
SSA00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDSA00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human SDF-1α Controls (optional; R&D Systems®, Catalog # QC20).

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.

Platelet-poor Plasma - Collect plasma on ice using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SDF-1α is a ligand for CXCR4. This receptor is expressed on cells of megakaryocytic lineage (9). Studies have shown that CXCR4 is present on platelets and has a high affinity for SDF-1 (10-12). Therefore, to measure circulating levels of SDF-1α, platelet-free plasma should be collected. It should be noted that many protocols for plasma preparation, including procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

Notes: *Serum samples are not recommended for use in this assay as platelet fragments present in serum can bind the SDF-1α in the sample.*
Grossly hemolyzed or icteric samples are not suitable for use in this assay.
Citrate plasma has not been validated for use in this assay.

SAMPLE PREPARATION

Use polyropylene tubes.

Cell culture supernate samples may require a 10-fold or greater dilution. A suggested 10-fold dilution is 25 µL of sample + 225 µL of Calibrator Diluent RD6Q.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

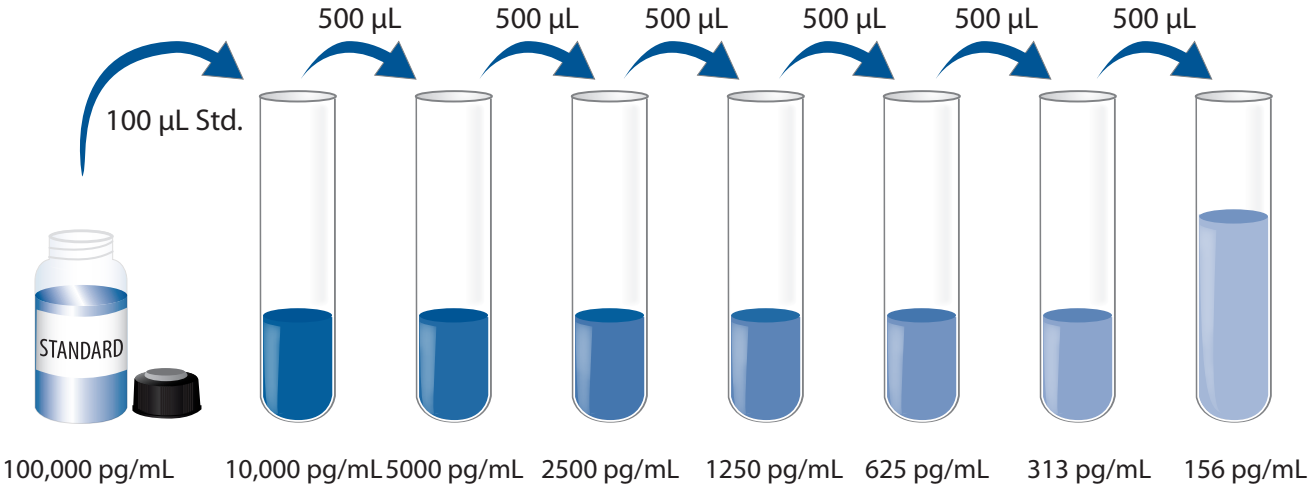
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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 SDF-1α Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human SDF-1α Standard with deionized or distilled water. This reconstitution produces a stock solution of 100,000 pg/mL. Mix the standard by inverting the vial 1-2 times to ensure complete reconstitution and allow the standard to sit for a minimum of 30 minutes prior to making dilutions.

Note: *Do not use rocker or vortex.*

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6Q into the 10,000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10,000 pg/mL standard serves as the high standard. Calibrator Diluent RD6Q serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples 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 Assay Diluent RD1-55 to each well.
4. Add 100 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12″ orbit) set at 500 ± 50 rpm.
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 SDF-1α Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

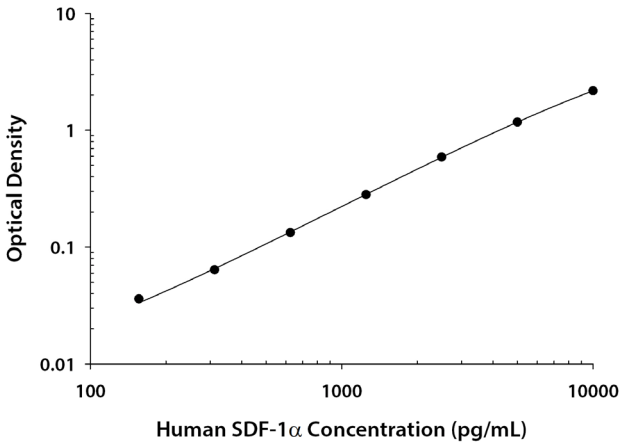
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the human SDF-1α concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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.



(pg/mL)	O.D.	Average	Corrected
0	0.025 0.025	0.025	—
156	0.061 0.061	0.061	0.036
313	0.089 0.089	0.089	0.064
625	0.158 0.158	0.158	0.133
1250	0.304 0.309	0.306	0.281
2500	0.606 0.624	0.615	0.590
5000	1.186 1.207	1.196	1.171
10,000	2.191 2.203	2.197	2.172

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 forty 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	40	40
Mean (pg/mL)	491	1700	3420	544	1600	3150
Standard deviation	19	58	115	73	150	257
CV (%)	3.9	3.4	3.4	13.4	9.4	8.2

RECOVERY

The recovery of SDF-1α spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	95-108%
Platelet-poor EDTA plasma (n=5)	97	90-107%
Platelet-poor heparin plasma (n=5)	97	90-104%

Note: *Because of the higher affinity of binding proteins or proteases for the recombinant protein and the lack of purified human SDF-1α, natural mouse SDF-1α was used for performing spike/recovery and linearity analyses.*

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of SDF-1α were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples* (n=4)	Platelet-poor EDTA plasma (n=4)	Platelet-poor heparin plasma (n=4)
1:2	Average % of Expected	103	107	107
	Range (%)	101-104	104-111	99-115
1:4	Average % of Expected	99	105	105
	Range (%)	97-99	103-108	102-110
1:8	Average % of Expected	96	106	107
	Range (%)	94-99	101-114	100-112

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Forty-one assays were evaluated and the minimum detectable dose (MDD) of human SDF-1α ranged from 1.0-47 pg/mL. The mean MDD was 18 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant human SDF-1α produced at R&D Systems®.

SAMPLE VALUES

Platelet-poor Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human SDF-1α in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Platelet-poor EDTA plasma (n=23)	2000	1360-2900
Platelet-poor heparin plasma (n=24)	1830	1330-2720

Cell Culture Supernates - ST-2 mouse bone marrow-derived stromal cells were grown in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were grown to confluence and were fed every 3-4 days for 4 weeks. An aliquot of the cell culture supernate was removed and assayed for levels of human SDF-1α. The sample was diluted 20-fold prior to assay and measured 51.7 ng/mL.

SPECIFICITY

This assay recognizes recombinant and natural human SDF-1α.

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 SDF-1α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
GROα
GROβ
GROγ
MCP-1
MIP-1α
MIP-1β
PTN
RANTES

Recombinant mouse:

MIP-1α
MIP-1β

Recombinant human SDF-1β exhibits 4.4% cross-reactivity in this assay.

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