Quantikine® ELISA

Mouse CXCL12/SDF-1α Immunoassay

Catalog Number MCX120

For the quantitative determination of mouse Stromal Cell-Derived Factor 1 alpha (SDF-1α) concentrations in cell culture supernates and serum.

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 non-ELR CXC chemokine that plays a role in a number of developmental and hematopoietic processes (1, 2). Unlike other non-ELR chemokines, SDF-1 is unusual in that its gene is located quite apart from the known genomic CXC chemokine cluster, and its receptor is monogamous relative to SDF-1 (1). In mice, there are two alternatively spliced variants of SDF-1. Mature SDF-1α is a 70 amino acid (aa) polypeptide, while SDF-1β has an identical, N-terminal 70 aa sequence with a 4 aa C-terminal extension (3, 4, 5). The significance of the C-terminal extension is unknown. In the brain, SDF-1β is associated with leukocyte trafficking while SDF-1α appears to modulate neuronal plasticity (6). SDF-1 is a highly basic, heparin-binding protein, and there is no evidence for glycosylation (2, 5). Truncated forms of SDF-1 are known to exist (5), with varying length deletions noted at the N-terminus (5, 7). CD26 is suggested to be partly responsible for the truncation which leads to bioinactivity (7). Cells known to express SDF-1 include neurons (SDF-1α) (6), fibroblasts (8, 9), thyrocytes (8), $CD34^+$ stem cells (10), thymic Hassel's corpuscles (11), vascular smooth muscle cells (9), and endothelial cells (6, 9, 12). Mouse to human, there are two aa differences over the mature segment of SDF-1β (97% identity) and one aa difference over the mature segment of SDF-1α (99% identity) (13). Mouse to rat, mature SDF-1α shows 96% aa identity (14).

SDF-1 binds to and activates the 42 kDa CXCR4 receptor (LESTR/fusin) (15). There are two alternate splice forms in mice, differing by two amino acids (15). Receptor activation involves dimerization, interaction with Gαi (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 (16).

SDF-1 has a number of activities. On hematopoietic progenitor cells, SDF-1 reportedly both chemoattracts progenitors and promotes their survival in the bone marrow and circulation (17-19). SDF-1 also has chemoattractant activity on NK cells, T cells, plasma cells and dendritic cells (20, 21). In the nervous system, SDF-1 attracts embryonic cerebellar external granular cells, and serves as a chemorepulsant for growing neuronal growth cones (22, 23). Finally, SDF-1 plays a crucial role in B cell lymphopoiesis, impacting the numbers of both pre- and pro-B cells in the bone marrow (24).

The Quantikine® Mouse CXCL12/SDF-1α Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse SDF-1α in cell culture supernates and mouse serum. It contains *E. coli*-expressed recombinant mouse SDF-1α and antibodies raised against the recombinant factor. It has been shown to accurately quantitate recombinant mouse 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 of natural mouse SDF-1α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse SDF-1α has been pre-coated onto a microplate. Standards, control, 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 mouse 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 mouse 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, 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.
- 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.
- 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
Mouse SDF-1α Microplate	890813	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse SDF-1α.	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.*
Mouse SDF-1α Standard	892404	2 vials of recombinant mouse SDF-1α in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Use within 2 hours of reconstitution. Discard after use.
Mouse SDF-1α Control	892410	2 vials of recombinant mouse SDF-1α in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the vial label.	
Mouse SDF-1α Conjugate	892403	11 mL of a polyclonal antibody specific for mouse 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	11 mL of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD6Q	895128	21 mL of animal serum 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 2N 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.
- 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.

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.

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 - Allow samples to clot for 2 hours at room temperature before centrifuging. Centrifuge for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Use polypropylene tubes.

Cell culture supernate samples require at least a 10-fold 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.

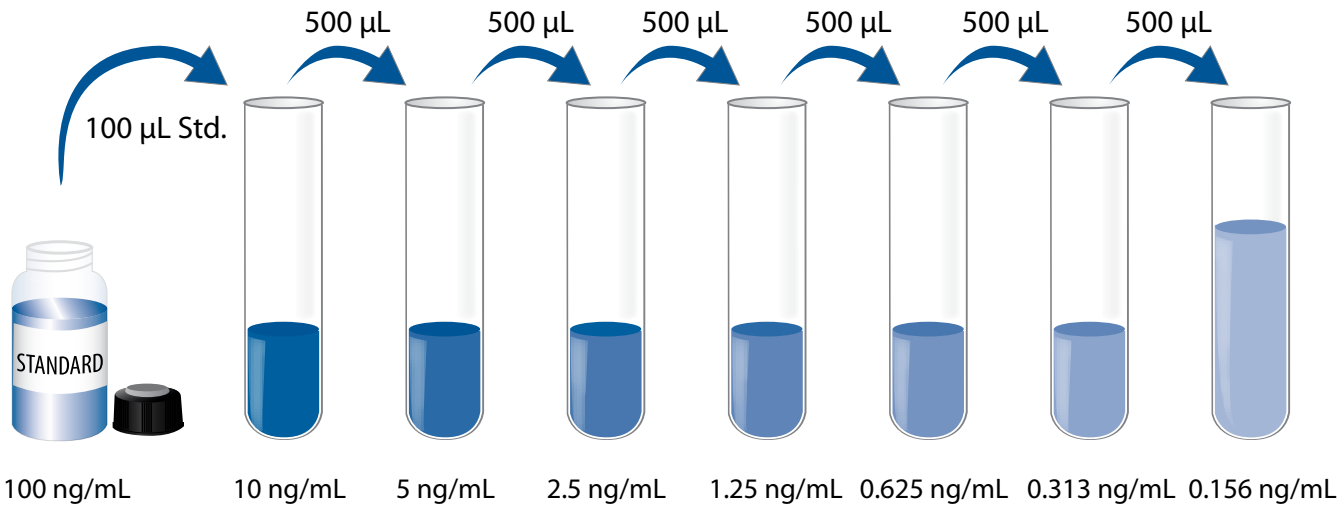
Mouse SDF-1α Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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.

Mouse SDF-1α Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse SDF-1α Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard gently to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6Q into the 10 ng/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 ng/mL standard serves as the high standard. Calibrator Diluent RD6Q serves as the zero standard (0 ng/mL).
Standard curve must be used within 30 minutes of preparation.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, control, 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 50 µL Assay Diluent RD1-55 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 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 100 µL of Mouse 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 if 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.

*Cell culture supernate samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

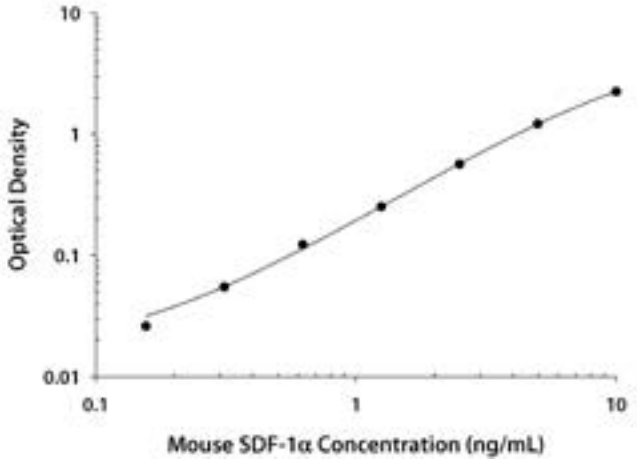
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D).

Create a standard curve by reducing the data using computer software capable of generating a 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 mouse SDF-1α 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 prior to assay, 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	0.060 0.060	0.060	——
0.156	0.082 0.089	0.086	0.026
0.313	0.114 0.116	0.115	0.055
0.625	0.181 0.184	0.183	0.123
1.25	0.311 0.312	0.312	0.252
2.5	0.618 0.630	0.624	0.564
5	1.261 1.285	1.273	1.213
10	2.281 2.337	2.309	2.249

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 assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	1.09	2.60	4.10	1.25	3.15	4.91
Standard deviation	0.04	0.12	0.21	0.09	0.24	0.37
CV (%)	3.7	4.6	5.1	7.2	7.6	7.5

RECOVERY

The recovery of mouse SDF-1α spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	98-104%

Note: *Recombinant protein cannot be used for spike/recovery and linearity analyses due to the higher affinity of binding proteins and proteases to the recombinant protein. 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 mouse SDF-1α were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernate* (n=1)	Cell culture media (n=4)	Serum (n=4)
1:2	Average % of Expected	102	99	100
	Range (%)	———	92-109	93-105
1:4	Average % of Expected	101	98	98
	Range (%)	———	92-107	96-100
1:8	Average % of Expected	94	99	98
	Range (%)	———	94-107	89-104
1:16	Average % of Expected	87	99	96
	Range (%)	———	88-112	90-102

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

SENSITIVITY

Twenty-six assays were evaluated and the minimum detectable dose (MDD) of mouse SDF-1α ranged from 0.014-0.069 ng/mL. The mean MDD was 0.044 ng/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 a highly purified *E. coli*-expressed recombinant mouse SDF-1α produced at R&D Systems®.

SAMPLE VALUES

Serum - Twenty-five samples were evaluated for the presence of mouse SDF-1α in this assay and ranged from 1.46-6.84 ng/mL. The mean was 3.26 ng/mL.

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 natural mouse SDF-1α. The sample measured 29.8 ng/mL.

SPECIFICITY

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

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the same factors at 100 ng/mL in a mid-range recombinant mouse SDF-1α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
BLC/BCA-1
GCP-2
IP-10 (CRG-2)
KC
MIG

Recombinant human:
ENA-78
BLC/BCA-1
GCP-2
GROα
GROβ
GROγ
IL-8
IP-10
I-TAC
MIG
NAP-2

Recombinant porcine:
IL-8

Recombinant human SDF-1α does not interfere but does cross-react approximately 26-42% in this assay.

Recombinant human SDF-1β does not interfere but does cross-react approximately 2.6% in this assay.

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