Quantikine® ELISA

Mouse SCF Immunoassay

Catalog Number MCK00

For the quantitative determination of mouse Stem Cell Factor (SCF) 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

Stem Cell Factor (SCF), also known as *c-kit* ligand (KL), mast cell growth factor (MGF), and Steel Factor (SLF), is a ligand for the *c-kit* tyrosine kinase receptor (1-4). Mutations in either SCF (localized to the *Sl* locus on mouse chromosome 10) or the *c-kit* tyrosine kinase receptor (localized to the *W* locus on mouse chromosome 5) have the same complex phenotype that affects hematopoiesis, gametogenesis and melanogenesis (1). *In vivo* and *in vitro*, SCF has potent effects on hematopoietic stem and progenitor cells (1, 5). SCF acts in a synergistic manner with various growth factors to induce myeloid, erythroid, megakaryocytic and lymphoid lineage cells. SCF synergizes with IL-2 and Flt-3 Ligand to stimulate the proliferation and cytotoxic activity of subsets NK cells. SCF also has marked activity on mast cells, inducing their growth, maturation, migration and activation (6-9).

Mouse SCF cDNA encodes a 248 amino acid (aa) residue type I transmembrane (TM) protein containing a 25 aa signal peptide, a 189 aa extracellular region, a 23 aa TM domain and a 36 aa cytoplasmic tail (1-4). Two alternately spliced isoforms of cell-associated SCF, KL-1, and KL-2, differing by the presence or absence of exon 6 that encode a proteolytic cleavage site, exist (10). The 164-165 aa secreted soluble form of SCF is generated by proteolytic cleavage from KL-1 (10, 11). The native soluble and cell-associated SCF are N- and O-glycosylated and both are biologically active (12, 13). Recombinant soluble SCF has been found to be non-covalently associated dimers *in vitro* (14). The dimer association constant is such however, that under physiological conditions, the majority of circulating SCF would exist as monomers. At the aa sequence level, mouse SCF is 95% and 80% identical to rat SCF and human SCF, respectively (11, 15, 16). Mouse SCF is widely expressed on different tissues and cells including fibroblasts, bone marrow stromal cells, macrophages, Sertoli cells, astroglia, and neurons (1, 5).

SCF receptor (*c-kit* tyrosine kinase receptor) is a type I membrane protein belonging to the type III tyrosine kinase growth factor receptor family, which also includes the M-CSF and PDGF receptors (1, 3, 4, 17, 18). This receptor family is characterized by the presence of five immunoglobulin-like domains in the extracellular region and a split receptor tyrosine kinase motif in the cytoplasmic region. SCF binding induces receptor homodimerization and signal transduction (1, 14). During embryogenesis and in the adult, SCF receptor is expressed in a variety of tissues and cells including hematopoietic progenitor cells, mast cells, germ cells, melanocytes, neurons, and glial cells (1, 5). SCF receptor can be proteolytically cleaved from the cell surface, and high levels of soluble SCF receptor have been detected in cell conditioned medium and plasma (5).

The Quantikine Mouse SCF Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse SCF levels in cell culture supernates, serum, and EDTA plasma. It contains *E. coli*-expressed recombinant mouse SCF and antibodies raised against the recombinant protein. Results obtained for naturally occurring mouse SCF 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 SCF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse SCF has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse SCF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse SCF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse SCF bound in the initial step. The sample values are then read off the standard curve.

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 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.
- For best results, pipette reagents and samples into the center of each well.
- 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.

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 SCF Microplate	890231	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse SCF.	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 SCF Standard	890241	2 vials of recombinant mouse SCF 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. Discard within 8 hours of reconstitution.
Mouse SCF Control	890230	2 vials of mouse SCF in a buffered protein base with preservatives; lyophilized. The assayed value of the Control should be within the range specified on the label.	
Mouse SCF Conjugate	890245	12 mL of a polyclonal antibody specific for mouse SCF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-54	895321	12 mL of animal serum with preservatives.	
Calibrator Diluent RD6-12	895214	21 mL of diluted 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	895174	23 mL of diluted hydrochloric 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.
- **Polypropylene** test tubes for dilution of standards and samples.

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging 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.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Heparin and citrate plasma have not been validated for use in this assay. Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-12.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

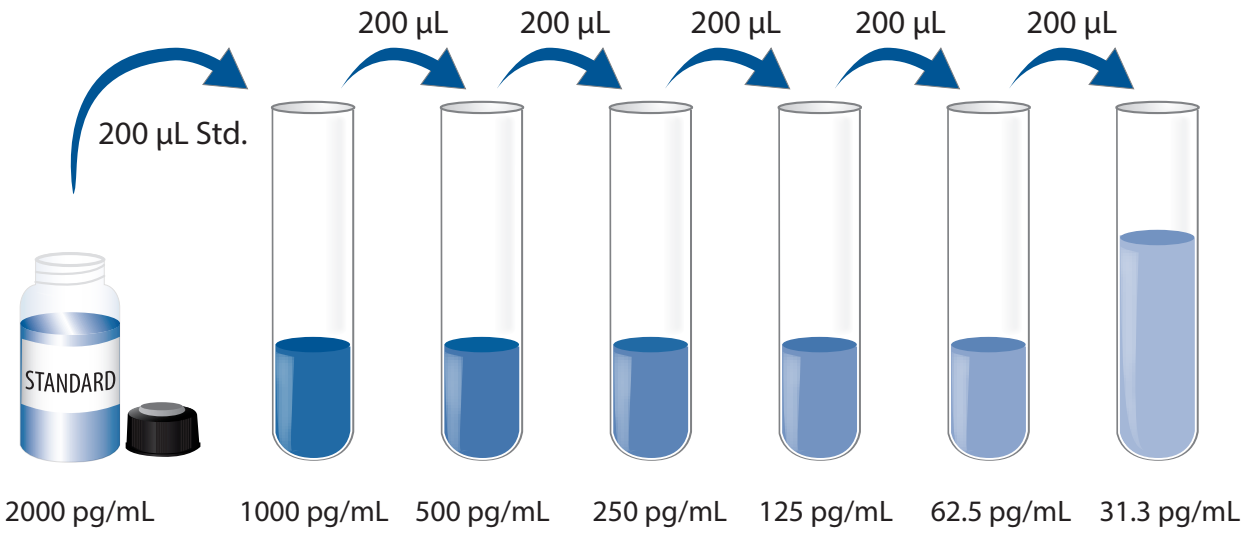
Mouse SCF Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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 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. 100 µL of the resultant mixture is required per well.

Mouse SCF Standard - **Refer to the vial label for reconstitution volume**. Reconstitute the Mouse SCF Standard with Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD6-12 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse SCF Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD6-12 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, Control, and samples be assayed in duplicate.

1. Prepare reagents, standard dilutions, Control, and samples* as directed by the previous section.
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 of Assay Diluent RD1-54 to each well.
4. Add 50 µL of Standard, Control, or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 SCF 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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

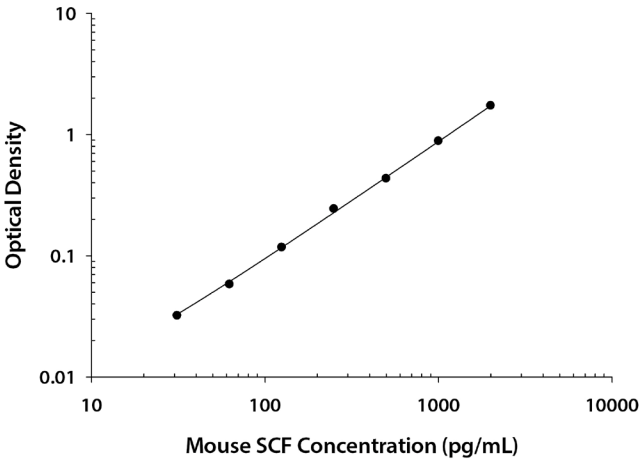
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 SCF 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.



(pg/mL)	O.D.	Average	Corrected
0	0.049 0.054	0.052	—
31.3	0.079 0.088	0.084	0.032
62.5	0.109 0.111	0.110	0.058
125	0.165 0.172	0.169	0.117
250	0.289 0.300	0.295	0.243
500	0.482 0.486	0.484	0.432
1000	0.933 0.934	0.934	0.882
2000	1.764 1.798	1.781	1.729

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 twenty 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	20	20	20
Mean (pg/mL)	67.2	183	1038	73.4	193	1033
Standard deviation	2.50	6.00	30.5	5.30	8.90	47.4
CV (%)	3.7	3.3	2.9	7.2	4.6	4.6

RECOVERY

The recovery of mouse SCF spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	105	99-113%
Serum* (n=5)	101	92-115%
EDTA plasma* (n=4)	102	84-113%

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

SENSITIVITY

The minimum detectable dose (MDD) of mouse SCF is typically less than 5 pg/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 mouse SCF produced at R&D Systems.

LINEARITY

To assess the linearity of the assay, five or more samples spiked with high concentrations of mouse SCF were diluted with Calibrator Diluent and then assayed.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Spiked	1104	———	———
	1:2	548	552	99
	1:4	266	276	96
	1:8	128	138	93
	1:16	65	69	94
Serum*	Spiked	1036	———	———
	1:2	501	518	97
	1:4	261	259	101
	1:8	124	130	95
	1:16	60	65	92
EDTA plasma*	Spiked	889	———	———
	1:2	446	444	100
	1:4	245	222	110
	1:8	122	111	110
	1:16	58	56	104

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

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of mouse SCF in this assay. The mean mouse SCF value was 141 pg/mL with a range of 108-212 pg/mL.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=40)	141	108-212
EDTA plasma (n=20)	159	114-218

SPECIFICITY

This assay recognizes natural and recombinant mouse SCF.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse SCF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10	IL-4	IL-18	PlGF-2
Eotaxin	IL-5	JE/MCP-1	RANTES
Flt-3 Ligand	IL-6	KC	TNF-α
G-CSF	IL-7	Leptin	TNF RI
GM-CSF	IL-9	LIF	TNF RII
IFN-γ	IL-10	MARC	Tpo
IL-1α	IL-10 R	MCP-5	VEGF
IL-1β	IL-12 p40	M-CSF	VEGF R1/Flt-1
IL-1ra	IL-12 p70	MIP-1α	
IL-2	IL-13	MIP-2	
IL-3	IL-17	OSM	

Recombinant human SCF cross-reacts approximately 5.1% in this assay.

REFERENCES

1. Lyman, S.D. and S.E. Jacobsen (1998) Blood **91**:1101.
2. Nocka, K. *et al.* (1990) EMBO J. **9**:3287.
3. Huang, E. *et al.* (1990) Cell **63**:225.
4. Williams, D.E. *et al.* (1990) Cell **63**:167.
5. Ashman, L.K. (1999) Int. J. Biochem. Cell Biol. **31**:1037.
6. Hogaboam, C. *et al.* (1998) J. Immunol. **160**:6166.
7. Nilsson, G. *et al.* (1994) J. Immunol. **153**:3717.
8. Hjertson, M. *et al.* (1996) Exp. Hematol. **24**:748.
9. Maurer, M. *et al.* (1998) J. Exp. Med. **188**:2343.
10. Huang, E.J. *et al.* (1992) Mol. Biol. Cell **3**:349.
11. Flanagan, J.G. *et al.* (1991) Cell **64**:1025.
12. Anderson, D.M. *et al.* (1990) Cell **63**:349.
13. Majumdar, M.K. *et al.* (1994) J. Biol. Chem. **269**:1237.
14. Hsu, Y-R. *et al.* (1997) J. Biol. Chem. **272**:6406.
15. Teramoto, T. *et al.* (1998) GenBank Accession # AF071204.1.
16. Martin, F.H. *et al.* (1990) Cell **63**:203.
17. Zsebo, K.M. *et al.* (1990) Cell **63**:213.
18. Qiu, F. *et al.* (1988) EMBO J. **7**:1003.