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ab176109 – Stem Cell Factor (SCF) Human SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of Stem Cell Factor (SCF) in human plasma, serum and cell culture supernatant samples.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Stem Cell Factor (SCF) *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of SCF protein in human plasma, serum and cell culture supernatant samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

SCF is the ligand for the receptor-type protein-tyrosine kinase KIT. SCF is expressed in fibroblasts, liver cells, Sertoli cells, endothelial cells, neurons, macrophages, oocytes, Schwann cells and numerous carcinoma cell lines. SCF can be found as a cell membrane protein or as a soluble protein after secretion due to proteolytic processing in the extracellular domain. Both forms have growth factor activities; however the membrane form is also important for cell-cell adhesion and interaction.

Soluble SCF is a heavily N- and O-glycosylated protein that exists as a non-covalently linked homodimer or as a heterotetramer with its receptor. Binding to the receptor, results in KIT activation by autophosphorylation. This leads to multiple signaling cascades (via

AKT, GRB2, RAS, MAPK amongst others) that are important in the regulation of cell survival, proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mast cell development, migration, melanogenesis and neurite-inductive activity of dorsal root ganglia neurons.

Genetic defects in SCF are the cause of familial progressive hyperpigmentation (melanosis universalis hereditaria). SCF has also been involved in other conditions such as mastocytosis, mast cell leukemia, myeloid leukemia, aplastic anemia and gastrointestinal stromal tumor.

2. ASSAY SUMMARY

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.

Add standard or sample to appropriate wells.

Add Antibody Cocktail to all wells. Incubate at room temperature.

Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X SCF Capture Antibody	600 µL	+2-8ºC
10X SCF Detector Antibody	600 µL	+2-8ºC
SCF Human Lyophilized Recombinant Protein	2 Vials	+2-8ºC
Antibody Diluent CPI2	6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
TMB Substrate	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50mL	+2-8ºC
Sample Diluent 50BP	20 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

Note: Antibody Diluent CPI2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CPI previously used in this kit.

While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit.

If you have any questions please contact Abcam Scientific Support.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail

Prepare Antibody Cocktail by diluting in the capture and detector antibodies in Antibody Diluent CPI2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the CSF standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the CSF standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 20 ng/mL **Stock Standard** Solution.

10.2 Label eight tubes numbers 1 – 8.

10.3 For **serum and plasma samples**, reconstitute the CSF standard by adding Sample Diluent 50BP. Add 380 µL Sample Diluent 50BP into tube #1 and 150 µL Sample Diluent 50BP into tube numbers 2-8.

Note: The Sample Diluent 50BP should be warmed to 37 ºC for 10 minutes and mixed thoroughly by inversion to ensure complete solubility, then equilibrated to room temperature before use.

For **cell culture supernatant samples**, reconstitute the CSF standard by adding non-reactive base media. Add 380 µL non-reactive base media into tube #1 and 150 µL non-reactive base media into tube numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range (%)**
Human Serum	1 - 50
Human Plasma - Citrate	1 - 50
Human Plasma - Heparin	1 - 50
Cell Culture Media	1 - 100

11.1 Plasma

Collect plasma using citrate or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles. Dilute samples to the desired concentration in Sample Diluent 50BP just before use.

Note: The Sample Diluent 50BP should be warmed to 37 ºC for 10 minutes and mixed thoroughly by inversion to ensure complete solubility, then equilibrated to room temperature before use.

11.2 Serum

Samples should be collected into a serum separator tube.

After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles. Dilute samples to the desired concentration in Sample Diluent 50BP just before use

11.3 **Cell Culture Supernatants**

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles. Dilute samples to the desired concentration in non-reactive base media just before use.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

12.3 Add 50 µL of each sample or standards to appropriate wells.

12.4 Add 50 µL of the Antibody Cocktail to each well.

12.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

12.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

12.7 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

12.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

12.9 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.059	0.058	0.059
7.8	0.106	0.107	0.106
15.6	0.136	0.133	0.134
31.3	0.212	0.208	0.210
62.5	0.349	0.343	0.346
125	0.631	0.640	0.635
250	1.108	1.075	1.091
500	1.951	1.982	1.966
1,000	3.357	3.360	3.358

Figure 1. Example of SCF standard curve in Sample Diluent NS. The SCF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.059	0.063	0.061
7.8	0.091	0.092	0.091
15.6	0.092	0.098	0.095
31.3	0.133	0.134	0.134
62.5	0.203	0.197	0.200
125	0.315	0.310	0.312
250	0.550	0.541	0.545
500	0.984	0.975	0.980
1,000	2.126	1.890	2.008

Figure 2. Example of SCF standard curve in Sample Diluent 50BP. The SCF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.065	0.064	0.065
15.6	0.133	0.144	0.123
31.3	0.178	0.147	0.163
62.5	0.232	0.227	0.229
125	0.444	0.393	0.419
250	0.814	0.708	0.761
500	1.468	1.393	1.430
1,000	2.461	2.511	2.486

Figure 3. Example of SCF standard curve in 100% cell culture media. The SCF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable (MDD) dose is 0.51 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentrations.

RECOVERY –

(Sample spiking in representative sample matrices)

Sample Type	Average % Recovery	Range (%)
100% Culture Media	106	100-113
50% Normal Human Serum	102	92-111
50% Normal Human Plasma - Citrate	89	79-103
50% Normal Human Plasma - Heparin	105	91-115

LINEARITY OF DILUTION –

Dilution Factor	Interpolated value	Human Serum 50%	Human Plasma (Citrate) 50%	Human Plasma (Heparin) 50%	Cell Culture Media 100%
Undiluted	pg/mL	557	514	576	565
	% Expected	**100**	**100**	**100**	**100**
2	pg/mL	266	261	280	250
	% Expected	**95**	**102**	**97**	**89**
4	pg/mL	121	125	138	135
	% Expected	**87**	**97**	**96**	**96**
8	pg/mL	67	53	66	64
	% Expected	**96**	**83**	**92**	**91**
16	pg/mL	32	26	32	-
	% Expected	**97**	**79**	**92**	**-**

PRECISION –

Mean coefficient of variations of interpolated values from pooled human serum samples within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	24	6
CV (%)	6	2

SAMPLE VALUES -

Figure 4. Frequency histogram of SCF levels in serum of individual normal healthy donors. The levels of SCF in serum samples were tested from ten individual healthy donors. Levels were interpolated from the standard curve in Sample diluent 50BP and corrected for sample dilution. The levels of SCF are shown for the percentage of individuals within each 100 pg/mL bin center of the distribution. The mean level of SCF was 330 pg/mL with a range of 185 to 498 pg/mL and a standard deviation of 82 pg/mL.

17. ASSAY SPECIFICITY

This kit detects Stem Cell Factor in human serum and plasma treated with sodium citrate or heparin only. Plasma EDTA is not recommended.

Human Cell and tissue extracts have not been tested with this kit. The immunogen for the capture and the detector antibodies used in this kit was full length human recombinant protein SCF, therefore we predict that this assay should be functional in these sample types.

18. SPECIES REACTIVITY

This kit recognizes human Stem Cell Factor protein.

Other species reactivity was determined by measuring 2X diluted serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Hamster
- Guinea Pig
- Rabbit
- Dog
- Goat
- Pig
- Cow

Please contact our Technical Support team for more information.

19. TROUBLESHOOTING

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

20. NOTES

Technical Support

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