



# KITLG (Human) ELISA Kit

Catalog Number KA1022

96 assays

Version: 04

Intended for research use only

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## **Introduction**

### **Background**

Stem Cell Factor (SCF) is also known as c-Kit receptor ligand, KL, steel factor, or mast cell growth factor. It is expressed in fibroblasts, thymus tissue, spleen, testes, placenta and mast cells. SCF is a cytokine that exists in two forms produced by alternative splicing: a soluble form of approximately 31 kDa and a membrane-bound form of approximately 32 kDa, lacking the proteolytic site for processing into the soluble form (1-4). SCF plays an important role in hematopoiesis, reproduction, and melanogenesis. It is also involved in proliferation and differentiation of mast cells. It stimulates mast cell activation in human bronchi and induces smooth muscle cell contraction (5).

### **Principle of the Assay**

The KITLG (Human) ELISA Kit is designed for detection of SCF in human plasma, serum and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human SCF in approximately 4 hours. A polyclonal antibody specific for human SCF has been pre-coated onto a 96-well microplate with removable strips. SCF in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human SCF, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## General Information

### Materials Supplied

List of component

Component	Amount
Human SCF Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human SCF.	96 (8x12) wells
Sealing Tapes: Each kit contains pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human SCF Standard: Human SCF in a buffered protein base (lyophilized).	128 ng
Biotinylated Human SCF Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against human SCF.	60 µL
EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	20 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	7 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction .	11 mL

### Storage Condition

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Store standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

### Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel)
- ✓ Deionized or distilled reagent grade water

**Precautions for Use**

- ✓ This product is for Research Use Only and is not intended for use in diagnostic procedures.
- ✓ Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acid solution.

## Assay Protocol

### Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ EIA Diluent Concentrate (10x): Dilute the EIA Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
- ✓ Human SCF Standard: Reconstitute the Human SCF Standard (128 ng) with 3.2 mL of EIA Diluent to generate a 40 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (40 ng/mL) 4-fold with EIA Diluent to produce 10, 2.5, 0.625, and 0.156 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[SCF] (ng/mL)
P1	1 part Standard (40 ng/mL)	40.00
P2	1 part P1 + 3 parts EIA Diluent	10.00
P3	1 part P2 + 3 parts EIA Diluent	2.500
P4	1 part P3 + 3 parts EIA Diluent	0.625
P5	1 part P4 + 3 parts EIA Diluent	0.156
P6	EIA Diluent	0.000

- ✓ Biotinylated Human SCF Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 100-fold with EIA Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- ✓ Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with EIA Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

### Sample Preparation

- ✓ Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 2-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).

- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 2-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.  
*Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.*
- ✓ Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A) 4 µL sample : 396 µL buffer (100x) = 100 fold dilution  Assuming the needed volume is less than or equal to 400 µL.	A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) = 10000 fold dilution  Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A) 4 µL sample : 396 µL buffer (100x) B) 24 µL of A : 216 µL buffer (10x) = 1000 fold dilution  Assuming the needed volume is less than or equal to 240 µL.	A) 4 µL sample: 396 µL buffer (100x) B) 4 µL of A : 396 buffer (100x) C) 24 µL of B : 216 µL buffer (10x) = 100000 fold dilution  Assuming the needed volume is less than or equal to 240 µL.

### **Assay Procedure**

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Human SCF Standard or sample to each well. Gently tap plate thoroughly coated the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually,

wash five times with 200  $\mu$ L of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300  $\mu$ L of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.

5. Add 50  $\mu$ L of Biotinylated Human SCF Antibody to each well. Gently tap plate thoroughly coated the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50  $\mu$ L of SP Conjugate to each well. Gently tap plate thoroughly coated the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50  $\mu$ L of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 8 minutes or until the optimal blue color density develops.
10. Add 50  $\mu$ L of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, substrate readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

✓ Assay Summary

1. Add 50  $\mu$ L of Standard or Sample per well. Incubate 2 hours.
2. Wash, then add 50  $\mu$ L of Biotinylated Antibody per well. Incubate 1 hour.
3. Wash, then add 50  $\mu$ L of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50  $\mu$ L of Chromogen Substrate per well. Incubate 8 minutes.
5. Add 50  $\mu$ L of Stop Solution per well. Read at 450 nm immediately.



## Data Analysis

### Calculation of Results

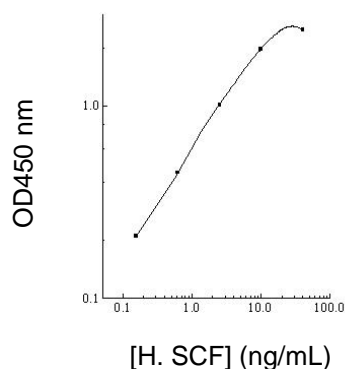
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.
- ✓ Typical Data

The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	40.0	2.289	2.287
		2.285	
P2	10.0	1.972	1.935
		1.897	
P3	2.50	1.109	1.107
		1.104	
P4	0.625	0.453	0.444
		0.434	
P5	0.156	0.213	0.211
		0.208	
P6	0.000	0.096	0.094
		0.092	
Sample: Pooled Normal Sodium Citrate Plasma (2x)		0.471	0.469
		0.467	

- ✓ Standard curve

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



- ✓ Reference Value
- Normal SCF plasma level range from 0.5-1.8 ng/mL.
- Plasma samples from healthy adults were tested (n=40). On average, human SCF level was 1.0 ng/mL.

### **Performance Characteristics**

- ✓ The minimum detectable dose of human SCF as calculated by 2SD from the mean of a zero standard was established to be 0.09 ng/mL.
- ✓ Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- ✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	5.3%	5.3%	4.1%	10.1%	9.7%	10.3%
Average CV (%)	4.9%			10.0%		

- ✓ Recovery

Standard Added Value	0.625-10 ng/mL
Recovery %	87-108%
Average Recovery %	95%

- ✓ Linearity

Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1x	96%	98%
2x	101%	99%
4x	104%	105%

- ✓ Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	< 10%
Mouse	None
Rat	None
Swine	None

## Resources

### Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	<ul style="list-style-type: none"> <li>• Check the expiration date listed before use.</li> <li>• Do not interchange components from different lots.</li> </ul>
	Improper wash step	<ul style="list-style-type: none"> <li>• Check that the correct wash buffer is being used.</li> <li>• Check that all wells are empty after aspiration.</li> <li>• Check that the microplate washer is dispensing properly.</li> <li>• If washing by pipette, check for proper pipetting technique.</li> </ul>
	Splashing of reagents while loading wells	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> </ul>
	Inconsistent volumes loaded into wells	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Insufficient mixing of reagent dilutions	<ul style="list-style-type: none"> <li>• Thoroughly agitate the lyophilized components after reconstitution.</li> <li>• Thoroughly mix dilutions.</li> </ul>
	Improperly sealed microplate	<ul style="list-style-type: none"> <li>• Check the microplate pouch for proper sealing.</li> <li>• Check that the microplate pouch has no punctures.</li> <li>• Check that three desiccants are inside the microplate pouch prior to sealing.</li> </ul>
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	<ul style="list-style-type: none"> <li>• Each step of the procedure should be performed uninterrupted.</li> </ul>
	Omission of step	<ul style="list-style-type: none"> <li>• Consult the provided procedure for complete list of steps.</li> </ul>
	Step performed in incorrect order	<ul style="list-style-type: none"> <li>• Consult the provided procedure for the correct order.</li> </ul>
	Insufficient amount of reagents added to wells	<ul style="list-style-type: none"> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Wash step was skipped	<ul style="list-style-type: none"> <li>• Consult the provided procedure for all wash steps.</li> </ul>
	Improper wash buffer	<ul style="list-style-type: none"> <li>• Check that the correct wash buffer is being used.</li> </ul>
	Improper reagent preparation	<ul style="list-style-type: none"> <li>• Consult reagent preparation section for the correct dilutions of all reagents.</li> </ul>
	Insufficient or prolonged incubation periods	<ul style="list-style-type: none"> <li>• Consult the provided procedure for correct incubation time.</li> </ul>

Deficient Standard Curve Fit	Non-optimal sample dilution	<ul style="list-style-type: none"> <li>• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay.</li> <li>• Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay.</li> <li>• User should determine the optimal dilution factor for samples.</li> </ul>
	Contamination of reagents	<ul style="list-style-type: none"> <li>• A new tip must be used for each addition of different samples or reagents during the assay procedure.</li> </ul>
	Contents of wells evaporate	<ul style="list-style-type: none"> <li>• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.</li> </ul>
	Improper pipetting	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Insufficient mixing of reagent dilutions	<ul style="list-style-type: none"> <li>• Thoroughly agitate the lyophilized components after reconstitution.</li> <li>• Thoroughly mix dilutions.</li> </ul>

### **References**

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**Plate Layout**

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2								
1								
	A	B	C	D	E	F	G	H