HUMAN SOLUBLE GLYCOPROTEIN OF 130 KDA (sgp 130) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SGP 130 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA

PRODUCT INFORMATION:

ELISA Name	**HUMAN sgp130 ELISA**
Catalog No.	SK00155-01
Lot No.	
Formulation	96 T
Standard range	78-10000 pg/mL
Sensitivity	39 pg/mL
Sample Volume	100 µl
Dilution factor	**50 (*Optimal dilutions should be determined by each laboratory for each application*)**
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human sgp 130
Calibration	Human sgp 130 Recombinant
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8 °C

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Human sgp130 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human sgp130 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human sgp130 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sgp130 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sgp130. The capture antibody can bind to the human sgp130 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human sgp130 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human sgp130 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

Description	Code	Quantity
sgp 130 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sgp 130.	**155-01-01**	**1 plate**
sgp 130 Standard – 10000pg/vial of recombinant human sgp 130 in a buffered protein base with preservatives; lyophilized.	**155-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrated of biotinylated antibody against sgp 130 with preservatives; lyophilized.	**155-01-03**	**1 vial**
Positive Control - one vial of recombinant human sgp 130, lyophilized	**155-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB01**	**2 bottles**
Antibody Diluent Solution Concentrate – 11mL of buffered protein based solution with preservatives	**DB20**	**1 tube**
HRP Diluent Solution – 12mL of buffered protein based solution with preservatives.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 8 months. For longer storage, unopened Standard, Positive Control, Detection Antibody Concentrate and Antibody Diluent Solution Concentrate should be

stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Detection Antibody concentrated solution and Antibody Diluent Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution (**protect from light**) and other components may be stored at 2 - 8°C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 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, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require a 50-fold dilution. A suggested 50-fold dilution is 5µL sample + 245 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.**

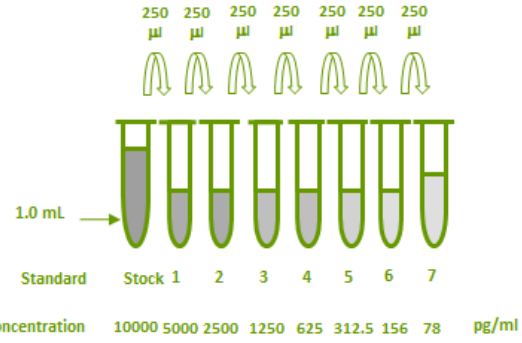
REAGENT PREPARATION

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. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

sgp 130 Standard - Reconstitute the sgp 130 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 10000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	Powder	1.0 ml	10000 pg/ml
# 1	250µl of stock	250µl	5000 pg/ml
# 2	250µl of 1	250µl	2500 pg/ml
# 3	250µl of 2	250µl	1250 pg/ml
# 4	250µl of 3	250µl	625 pg/ml
# 5	250µl of 4	250µl	312.5 pg/ml
# 6	250µl of 5	250µl	156 pg/ml
# 7	250µl of 6	250µl	78 pg/ml



Antibody Diluent Solution Concentrate – Reconstitute the Antibody Diluent Solution Concentrate with 11.0 mL of Dilution Buffer in provided 15 mL tube to prepare Antibody Diluent Solution.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB20)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of

Antibody Diluent Solution into another 15 ml centrifuge tube and transfer the 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note: Must be prepared 1 to 2 hours prior to use.**

Streptavidin-HRP Conjugate - Pipette 11.94 mL of **HRP Diluent Solution (DB08)** into a 15 ml centrifuge tube and transfer 60 µl of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light).**

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control solution. **Note:** Positive Control could be used within a few days if stored at -20 °C or -70 °C.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells.
4. Add 100 µL of **Standard dilutions**, **sample**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. **Prepare Detection Antibody working solution.**
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 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 **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 1-10 minutes on micro-plate shaker at room temperature. **Protect from light.**
11. Add 100 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.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

Protein	Cross-reactivity (%)
Human sgp 130	100
Mouse sgp 130	0
Human CNTF	0
Human IL-6	0
Human IL-11	0

LINEARITY

To assess the linearity of the assay, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
50X	3847	192350	100
100X	2053	205300	107

To assess the linearity of the assay, pooled research human **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
50X	4079	203950	100
100X	2396	239600	117

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.068)
78	0.028
156	0.058
312.5	0.127
625	0.235
1250	0.450
2500	0.785
5000	1.395
10000	2.134

- Lot No.:
- Positive Control:

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
⇩
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT. **Prepare Detection Antibody working solution.**
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate Solution to each well. Incubate 1-10 min on the plate shaker at RT. **Protect from light.**
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.