Quantikine® ELISA

Human soluble gp130 Immunoassay

Catalog Number DGP00

For the quantitative determination of recombinant human soluble gp130 (sgp130) concentrations in cell culture supernates, serum, and plasma.

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

Interleukin 6 (IL-6) is a multifunctional protein that plays important roles in host defense, acute phase reactions, immune responses, and hematopoiesis (1, 2). IL-6 exerts its activity through binding to a high affinity receptor complex consisting of two membrane glycoproteins: an 80 kDa IL-6-binding receptor protein (IL-6 R) (3, 4) and a 130kDa signal-transducing protein (gp130) (5, 6). Human gp130 is made up of a 597 amino acid (aa) residue ectodomain, a 22 aa residue transmembrane domain and a 277 aa residue cytoplasmic domain (7). Murine gp130 is approximately 77% identical to human gp130 at the amino acid sequence level. The ectodomain of gp130 is made up of a cytokine receptor superfamily domain as well as an immunoglobulin-like domain and three fibronectin type III-like domains. The cytoplasmic domains of neither IL-6 R nor gp130 has homology with known tyrosine kinases or other catalytic domains (1).

IL-6 has been shown to bind to IL-6 R with low-affinity (3, 4). In the absence of IL-6 R, IL-6 does not appear to bind to gp130 (5, 6), however, the presence of IL-6 R together with gp130 will result in high-affinity IL-6 binding and subsequent signal transduction (8, 9). In addition to serving as the signal transducing and high-affinity-converting subunit for the IL-6 receptor complex, gp130 has been shown to be a signal transducer for several other cytokines with overlapping biological functions, including IL-11, LIF, OSM, CNTF, and Cardiotrophin-1 (CT-1) (8, 10-14). With the exception of OSM and LIF, which bind gp130 with low-affinity, the other cytokines do not appear to bind gp130 in the absence of additional receptor subunits (15). Gp130 has been shown to be expressed in nearly all human and mouse cell lines examined (5, 6). A current model for IL-6 signal transduction assumes that one IL-6 molecule binds an IL-6 R molecule forming an IL-6/IL-6 R complex. The formation of this complex induces high-affinity binding to and dimerization of gp130 (involving disulfide links between two gp130 molecules) (16). Dimerization of gp130 initiates signal transduction via the JAK/STAT pathway (17-21). IL-6 stimulation has also been shown to activate RAS and mitogen-activated protein kinases (MAPK) that are essential for NF-IL-6 (a nuclear factor essential for IL-6 expression) activation (22). Based on results of size-exclusion and analytical ultracentrifugation analysis of the interaction of IL-6, soluble IL-6 R and soluble gp130, it has been proposed that the high-affinity IL-6 receptor complex is a hexameric complex consisting of two molecules each of IL-6, IL-6 R and gp130 (23).

A naturally occurring soluble form of the IL-6 R, which binds IL-6 and which mediates IL-6 signaling through interaction with gp130, has been detected in the urine of healthy adult humans (24), in human and mouse serum, and in the conditioned medium of various cells, including human peripheral blood mononuclear cells, and several T cell or granulocyte/macrophage cell lines (25-27). A naturally occurring soluble form of gp130 has also been found in human serum (28) and in the ascites fluid of tumor-bearing mice (28). Soluble gp130 has been shown to be capable of inhibiting gp130-mediated cytokine activities *in vitro*. Both the soluble IL-6 R and the soluble gp130 are present in nanogram quantities in the serum of normal individuals. These observations suggest that a complex regulatory system is involved in the modulation of the biological activities of IL-6 under normal and pathological conditions.

The Quantikine Human soluble gp130 Immunoassay is a 4.5-5.5 hour solid phase ELISA designed to measure sgp130 in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human gp130 and antibodies raised against recombinant human gp130 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural gp130 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 for natural sgp130.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human sgp130 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sgp130 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human sgp130 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 sgp130 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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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
Human sgp130 Microplate	890294	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human sgp130.	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.*
Human sgp130 Standard	890296	80 ng of recombinant human sgp130 in a buffered protein base with preservatives; lyophilized.	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.*
Human sgp130 Conjugate	890295	21 mL of polyclonal antibody specific for human sgp130 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a buffered protein base with preservatives. *Use undiluted for cell culture supernate samples. Use diluted 1:5 for serum and plasma samples.*	
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 2 N 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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human sgp130 Controls (optional; available from R&D Systems).

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 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 at room temperature 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.

Note: *Grossly lipemic samples are not suitable for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require at least a 100-fold dilution in Calibrator Diluent RD5P (diluted 1:5)* prior to the assay. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5).

*See Reagent Preparation section.

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. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

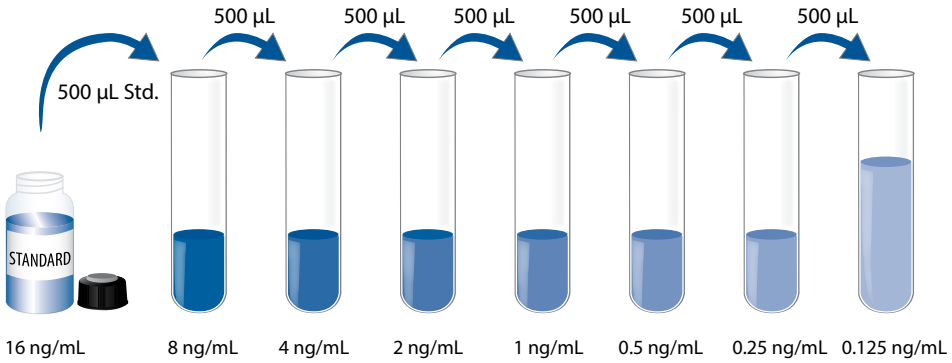
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P Concentrate for Cell Culture Supernate Samples - **DO NOT** dilute Calibrator Diluent RD5P Concentrate.

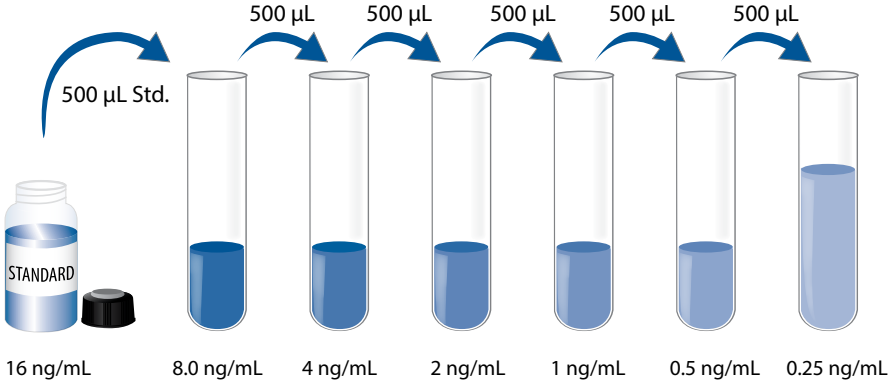
Calibrator Diluent RD5P (diluted 1:5) for Serum/Plasma Samples - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to yield 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human sgp130 Standard - Reconstitute the Human sgp130 Standard with 5.0 mL of Calibrator Diluent RD5P Concentrate (*for cell culture supernate samples*) or Calibrator Diluent RD5P (diluted 1:5) (*for serum/plasma samples*). This reconstitution produces a stock solution of 16 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Use this stock solution to produce a dilution series (below).

For Cell Culture Supernate Samples - Pipette 500 µL of Calibrator Diluent RD5P Concentrate into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8 ng/mL standard serves as the high standard. Calibrator Diluent RD5P Concentrate serves as the zero standard (0 ng/mL).



For Serum/Plasma Samples - Pipette 500 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. The undiluted standard (16 ng/mL) serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, standards, 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 100 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of Standard, samples*, or control per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature.
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 200 µL of Human sgp130 Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernates: Incubate for 2 hours at room temperature.
   For Serum/Plasma: Incubate for 1 hour at room temperature.
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. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the well should change from blue to yellow. If the color in the well 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.

*Serum/plasma 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.).

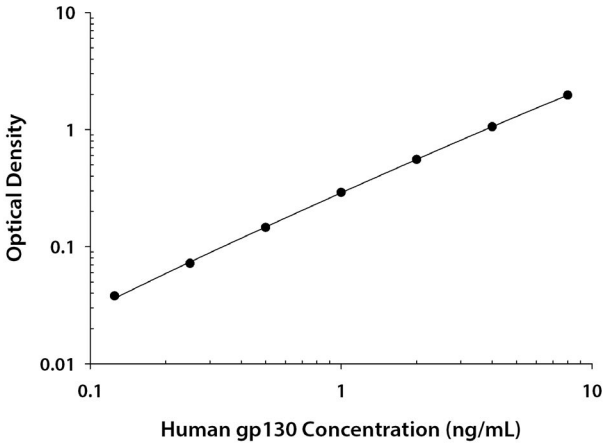
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human sgp130 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

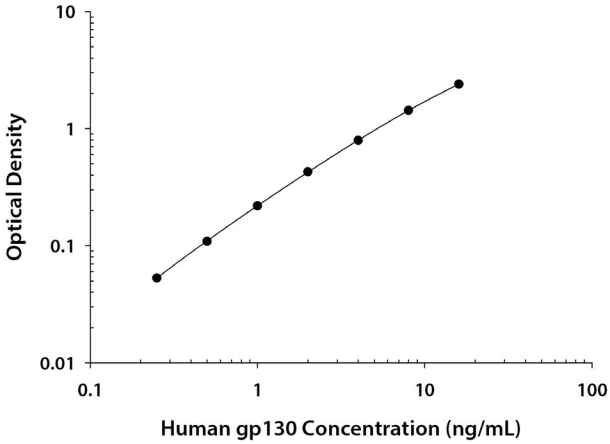
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.014 0.014	0.014	—
0.125	0.052 0.052	0.052	0.038
0.25	0.086 0.086	0.086	0.072
0.5	0.159 0.161	0.160	0.146
1.0	0.306 0.302	0.304	0.290
2.0	0.574 0.567	0.570	0.556
4.0	1.073 1.071	1.072	1.058
8.0	1.973 1.983	1.978	1.964

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
0.25	0.064 0.062	0.063	0.053
0.5	0.119 0.119	0.119	0.109
1.0	0.228 0.230	0.229	0.219
2.0	0.436 0.441	0.438	0.428
4.0	0.805 0.798	0.802	0.792
8.0	1.430 1.448	1.439	1.429
16.0	2.398 2.404	2.401	2.391

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 separate assays to assess inter-assay precision.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.40	1.00	4.43	0.40	1.02	4.50
Standard deviation	0.02	0.07	0.33	0.02	0.10	0.31
CV (%)	5.0	7.0	7.4	5.0	9.8	6.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.70	1.82	8.43	0.77	1.94	8.64
Standard deviation	0.03	0.10	0.40	0.04	0.07	0.30
CV (%)	4.3	5.5	4.7	5.2	3.6	3.5

RECOVERY

The recovery of human sgp130 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	109	96-114%
Serum (n=5)	101	83-120%
EDTA plasma (n=5)	100	83-114%
Heparin plasma (n=5)	98	85-110%
Citrate plasma (n=5)	98	84-112%

SENSITIVITY

Using Calibrator Diluent RD5P Concentrate the minimum detectable dose (MDD) of sgp130 was found to be less than 0.05 ng/mL. Using Calibrator Diluent RD5P (diluted 1:5) the MDD was found to be less than 0.08 ng/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.

LINEARITY

To assess linearity of the assay, samples were spiked with high concentrations of human sgp130 and diluted with the appropriate Calibrator Diluent to produce sample values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)
1:2	Average % of Expected	105	102	103	101	106
	Range (%)	102-106	99-104	99-105	90-110	100-115
1:4	Average % of Expected	100	103	104	103	105
	Range (%)	96-104	102-105	98-111	101-107	101-107
1:8	Average % of Expected	97	102	103	101	107
	Range (%)	93-101	100-104	98-106	98-105	101-112
1:16	Average % of Expected	96	101	105	100	104
	Range (%)	89-102	96-104	100-110	93-108	98-108

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human sgp130 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human sgp130 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=60)	305	238-372	28
EDTA Plasma (n=60)	267	214-360	30
Heparin Plasma (n=60)	267	190-337	35
Citrate Plasma (n=60)	230	176-294	24

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human sgp130.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	0.85	N/A
Stimulated	1.01	1.20

SPECIFICITY

This assay recognizes natural and recombinant human sgp130.

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 rhsgp130 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CNTF
G-CSF
G-CSF R
GM-CSF
IL-6
IL-6 Rα
IL-11
LIF
LIF Rα
M-CSF
OSM

Recombinant mouse:

IL-6

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