



## **DRG<sup>®</sup> Human sgp130 / sCD130 ELISA (EIA-4768)**

**As of 19 Feb. 2008**

**RUO**

### **INTENDED USE**

The Human sGP130 ELISA is to be used for the in-vitro quantitative determination of gp130 protein (sGP130) in human serum, plasma, buffered solutions or cell culture medium. The assay will recognize both natural and recombinant human sGP130.

**This kit has been configured for research use only and is not to be used in diagnostic procedures.**

### **PRINCIPLE OF THE METHOD**

The sGP130 Kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA).

A monoclonal antibody specific for GP130 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known sGP130 concentrations, control specimens and unknowns are pipetted into these wells. During the first incubation, the sGP130 antigen and a biotinylated monoclonal antibody specific for GP130 are simultaneously incubated. After washing, the enzyme (streptavidin-peroxydase) is added. After incubation and washing to remove all the unbound enzyme, a substrate solution which is acting on the bound enzyme is added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of sGP130 present in the samples.

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**REAGENTS PROVIDED AND RECONSTITUTION**

REAGENTS (Store at 2-8°C)	QUANTITY	RECONSTITUTION
96-wells microtiter plate	1	Ready-to-use
Plastic cover	2	
Standard : 1800 pg/mL	2 Vials Freeze Dried	1.4 mL of adequate standard buffer diluent for 1 vial
Control : 384+/-63 pg/mL	2 Vials Freeze Dried	0.5 mL of adequate standard buffer diluent for 1 vial
Standard Buffer Diluent	1 Vial (25 mL)	Dilute in distilled Water
Biotinylated anti-GP130	1 Vial (0.3 ml)	Dilute in biotinylated antibody diluent
Biotinylated Antibody Diluent	1 Vial (13 mL)	Ready-to-use
Streptavidin-HRP	2 Vials (5 µL)	0.5 mL of HRP-Diluent before further dilutions
HRP Diluent	1 Vial (23 mL)	Ready-to-use
Washing Buffer	1 Vial (10 mL)	Dilute in distilled Water
Chromogen TMB :	1 Vial (21 mL)	Ready-to-use
Stop Reagent, H <sub>2</sub> SO <sub>4</sub> :	2 Vials (11 mL)	Ready-to-use

**MATERIAL REQUIRED BUT NOT PROVIDED**

- Distilled water.
- Pipettes : 10 µL, 50 µL, 100 µL, 200 µL and 1000 µL.
- Vortex mixer and magnetic stirrer.

**SAFETY**

- For research use only.
- The human blood components included in this kit have been tested and found non reactive for HBsAg and anti-HIV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Avoid any skin contact with H<sub>2</sub>SO<sub>4</sub> and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette with mouth.

**PROCEDURAL NOTES/LAB. QUALITY CONTROL**

- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilized standards and controls should be discarded right after resuspension and use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different kit lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, control or specimen addition in order to avoid cross-contamination ; for the dispensing of H<sub>2</sub>SO<sub>4</sub> and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- Control should be run with each assay. If control values read outside pre-established ranges, the accuracy of the assay is suspect.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- Serum and plasma samples should be collected in pyrogen/endotoxin-free tubes.
- Samples should be stored frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen specimens. Thaw completely prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, this should be removed prior to assay by centrifugation or filtration.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Respect incubation times described in the assay procedure.
- Dispense the TMB solution within 15 min. following the washing of the microtiter plate.

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**RUO****REAGENTS PREPARATIONS****Standard buffer diluent**

Dilute 5 mL of standard buffer diluent in 50 mL of distilled water or all the content of the vial (25 mL) in 250 mL of distilled water.

**Standards and Controls**

Standard have to be reconstituted with 1.4 mL of adequate standard buffer diluent to give a concentration of 1800 pg/mL sGP130.

Controls should be resuspended in 0.5 mL of adequate standard buffer diluent.

**Human serum samples**

Before testing, human serum samples have to be diluted 200 times in standard buffer diluent.

**Dilution of biotinylated anti-sGP130**

Dilute the biotinylated anti-sGP130 with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette. Extemporaneous preparations are recommended.

Number of Wells used	Biotinylated Antibody (µL)	Biotinylated Antibody Diluent (µL)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

**Dilution of Streptavidin-HRP**

Add 0.5 mL of HRP diluent to a 5 µL vial of Streptavidin-HRP. DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS. Extemporaneous preparations are recommended. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial : see hereafter the table for volumes to pipette.

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Number of Wells	Streptavidin-HRP (µL)	Strep-HRP Diluent (mL)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

## **Dilution of Washing Buffer**

Dilute 2 mL of washing buffer in 400 mL of distilled water or all the content of one vial (10 mL ) in 2000 mL of distilled water.

## **ASSAY METHOD**

1. Before use, mix all reagents thoroughly without making foam.
2. Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control samples should be assayed in duplicate. Remove sufficient microwell strips from the pouch.
3. Add 100 µL of appropriate standard diluent to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation.

Pipet 200 µL of standard into wells A1 and A2 (see Plate Scheme below).

Transfer 100 µL from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells.

Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of sGP130 standard dilutions ranging from 1800 to 56.25 pg/mL.

Discard 100 µL from the content of the last microwells used (F1, F2).

4. Add 100 µL of appropriate standard diluent to the blank wells ( G1-G2 ).
5. Add 100 µL of samples to sample wells and 100 µL of control to the control wells (H1, H2).
6. Preparation of biotinylated anti-GP130 : see reagents preparation.
7. Add 50 µL of diluted biotinylated anti-GP130 to all wells.
8. Cover with a plate cover and incubate for 1 hour at room temperature (18°C - 25°C ).
9. Remove the cover plate and wash the plate as follows:
  - a) aspirate the liquid from each well ;
  - b) dispense 0.3 mL of washing solution into each well ;
  - c) aspirate again the content of each well ;
  - d) Repeat steps b) and c) two times.
10. Prepare HRP solution just before use: see reagents preparation.
11. Dispense 100 µL of HRP solution into all wells, including the blank wells. Put back the cover plate.

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12. Incubate the microwell strips at room temperature for 30 minutes.
13. Remove plate cover and empty wells. Wash microwell strips according to point (i). Proceed immediately to the next step.
14. Pipette 100 µL of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for 12-15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
15. Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable (maximum 20 minutes).
16. The enzyme-substrate reaction is stopped by quickly pipetting 100 µL of 1.8 N sulfuric acid into each well, including the blank wells, to completely and uniformly inactivate the enzyme.  
Results must be read immediately after the addition of sulfuric acid, or within one hour, if the microwell strips are stored at 2-8°C in the dark.
17. Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

### SUGGESTED PLATE SCHEME

	Standard Conc. pg/mL		Sample wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	1800	1800										
B	900	900										
C	450	450										
D	225	225										
E	112.5	112.5										
F	56.25	56.25										
G	Blank	Blank										
H	Ctrl	Ctrl										

### DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding sGP130 standard concentration on the horizontal axis. The amount of sGP130 in each sample is determined by extrapolating OD values to sGP130 concentrations using the standard curve.

### LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 1800 pg/mL standard curve point.

The dose-response is non-linear in this region and good accuracy is difficult to obtain.

DRG International Inc., USA

Fax: (908) 233-0758 • E-mail: [corp@drg-international.com](mailto:corp@drg-international.com) • Web: [www.drg-international.com](http://www.drg-international.com)

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Concentrated samples (> 1800 pg/mL) have to be diluted with standard diluent or with your own sample buffer. During analysis, multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) and the use of plasma instead of serum specimens has not been thoroughly investigated.

The rate of degradation of native sGP130 in various matrices has not been investigated.

The immunoassay literature contains frequent references to aberrant signals observed with some human sera and attributed to heterophilic (or anti-mouse) antibodies. Though we have not found such specimens to date, the possibility of this occurrence can not be excluded.

### PERFORMANCES AND CHARACTERISTICS

#### 1.1 Sensitivity

The minimum detectable dose of sGP130 is less than 15 pg/mL.

This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 32 times.

#### 1.2 Precision

Intra-Assay					Inter-Assay				
Sample	n	Mean(ng/mL)	SD	CV%	Sample	n	Mean(ng/mL)	SD	CV%
A	8	1751	17.61	1.00	A	16	1782	26.64	1.49
B	8	264.3	8.67	3.27	B	16	247.0	11.99	4.85

#### 1.3 Linearity of Dilution

A human serum pool containing 110 ng/mL of measured gp130 is serially diluted in standard buffer diluent over the range of the assay. Linear regression of sample versus the expected concentration yield a correlation coefficient of 0.99.

#### 1.4 Recovery

Recovery of gp130 added to pooled human serum is 97% for concentrations comprised within the range of the assay.

#### 1.5 Normal Serum values

The average concentration of sGP130 detected in 80 normal human serum samples is  $148 \pm 34$  ng/mL ranging from 43 to 219 ng/mL.

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## ASSAY PROCEDURE SUMMARY

Total procedure length : 1h45

Add 100 µL of sample or diluted standard or control

Add 50 µL of diluted biotinylated detection antibody to all wells

Incubate 1 hour at room temperature

Add 100 µL of HRP-Streptavidin conjugate











Incubate 30min at room temperature


Add 100 µL of ready-to-use TMB Protect from light. Let the color develop for 10-15 min.

Add 100 µL H<sub>2</sub>SO<sub>4</sub>

Read Absorbance at 450 nm

## SYMBOLS USED WITH DRG ASSAY'S

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
	Conformidade com as normas europeias	Europeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση

DRG International Inc., USA




Fax: (908) 233-0758 • E-mail: [corp@drg-international.com](mailto:corp@drg-international.com) • Web: [www.drg-international.com](http://www.drg-international.com)



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<b>IVD</b>	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
<b>RUO</b>				
<b>REF</b>	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
<b>LOT</b>	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..