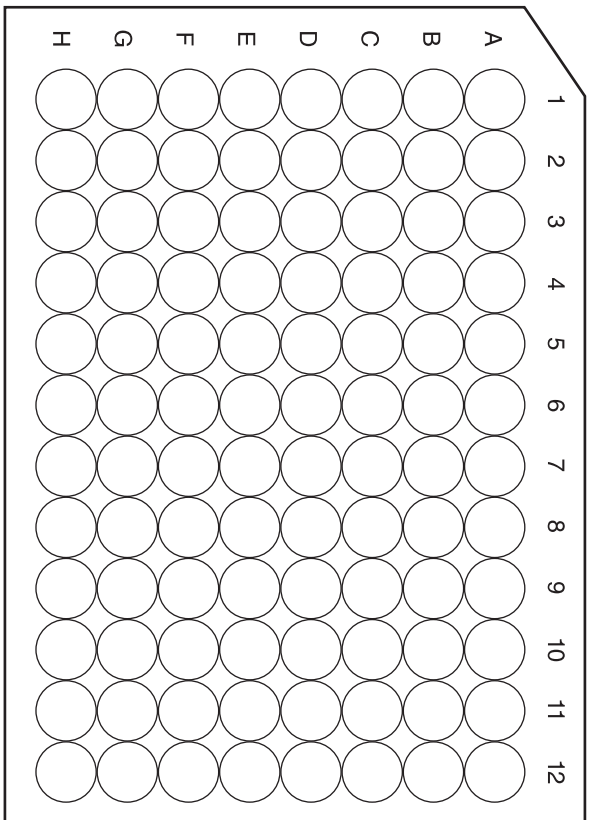

Human HPRG Immunoassay

Catalog Number: SEKH-0419

For the quantitative determination of human HPRG concentrations in cell culture supernates,serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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TABLE OF CONTENTS

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of HPRG in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	86	97
	Range(%)	80-94	90-103
1:4	Average% of Expected	94	102
	Range(%)	88-102	96-110

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15.63 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant human HPRG. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

RECOVERY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of HPRG spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of HPRG in two matrices

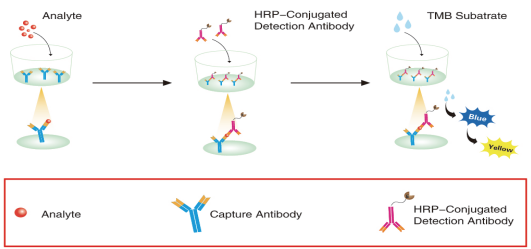
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	94	85-103
Cell culture supernatants	96	86-105

BACKGROUND

Histidine-rich glycoprotein, also known as HRG and HPRG, is a glycoprotein located in plasma and platelets, and contains an unusually large amount of histidine and proline. In human, five distinct domains are recognized in the mature HPRG molecule. There are two N-terminal cystatin-like modules (aa 19 - 254) and one His-Pro-rich region (aa 350 - 497) that is flanked by two Pro-rich segments (aa 276 - 321 and 498 - 525). The His-Pro-rich region contains 10 tandem repeats with an HHPHG motif, and the N- and C-termini are linked by a disulfide bond. The specific functions of HRG remain unclear, but it is known that the protein binds heme, dyes and divalent metal ions. It inhibits rosette formation and interacts with heparin, thrombospondin and plasminogen.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific forHPRG/HRG has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and anyHPRG/HRG present is captured by the coated antibody after incubation. Following extensive washing, a Detection antibody specific forHPRG/HRG is added to detect the capturedHPRG/HRG protein in sample, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

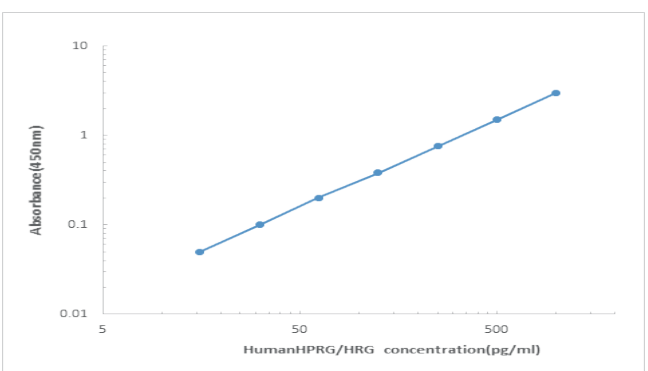
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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

Typical data using the HPRG ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.041	0.043	0.042	--------
15.62	0.093	0.089	0.091	0.049
31.25	0.147	0.145	0.146	0.104
62.5	0.234	0.240	0.237	0.195
125	0.407	0.412	0.410	0.368
250	0.719	0.723	0.721	0.679
500	1.265	1.267	1.266	1.224
1000	2.387	2.393	2.390	2.348



Representative standard curve for HPRG ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed.

⇩

Add 100µl standard or samples to each well, incubate 120 minutes, at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Detection antibody to each well, incubate 60 minutes,at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-30 minutes,at room temperature(25±2℃)..Protect from light.

⇩

Add 50ul Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r or so

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the HPRG concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	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℃**
Standard - lyophilized,1000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Detection antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Detection antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.
6.Human HPRG controls (optional; available from Solarbio).

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. 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 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

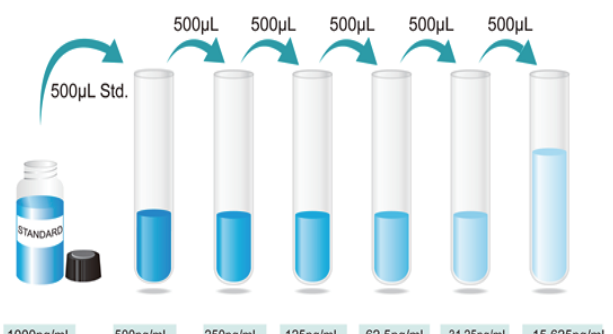
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning -** Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer -** Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard\Sample -** Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.Pipette 500μL of Standard/Sample Diluent into 500pg/ml tube and the remaining tubes. Use the stock solution of 1000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1000 pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of Detection antibody:** Make a 1:100 dilution of the concentrated Detection Antibody with the Detection Antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**



Preparation of HPRG standard dilutions