



Human HRG / HPRG ELISA Kit

Enzyme Immunoassay for the quantification of Human HRG / HPRG in Human Serum, plasma (EDTA, heparin) and cell culture supernatants.

Catalog number: ARG82374

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

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INTRODUCTION

This histidine-rich glycoprotein contains two cystatin-like domains and is located in plasma and platelets. The physiological function has not been determined but it is known that the protein binds heme, dyes and divalent metal ions. The encoded protein also has a peptide that displays antimicrobial activity against *C. albicans*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *E. faecalis*. It can inhibit rosette formation and interacts with heparin, thrombospondin and plasminogen. Two of the protein's effects, the inhibition of fibrinolysis and the reduction of inhibition of coagulation, indicate a potential prothrombotic effect. Mutations in this gene lead to thrombophilia due to abnormal histidine-rich glycoprotein levels. [provided by RefSeq, Nov 2014]

Plasma glycoprotein that binds a number of ligands such as heme, heparin, heparan sulfate, thrombospondin, plasminogen, and divalent metal ions. Binds heparin and heparin/glycosaminoglycans in a zinc-dependent manner. Binds heparan sulfate on the surface of liver, lung, kidney and heart endothelial cells. Binds to N-sulfated polysaccharide chains on the surface of liver endothelial cells. Inhibits rosette formation. Acts as an adapter protein and is implicated in regulating many processes such as immune complex and pathogen clearance, cell chemotaxis, cell adhesion, angiogenesis, coagulation and fibrinolysis. Mediates clearance of necrotic cells through enhancing the phagocytosis of necrotic cells in a heparan sulfate-dependent pathway. This process can be regulated by the presence of certain HRG ligands such as heparin and zinc ions. Binds to IgG subclasses of immunoglobins containing kappa and lambda light chains with different affinities regulating their clearance and inhibiting the formation of insoluble immune complexes. Tethers plasminogen to the cell surface. Binds T-cells and alters the cell morphology. Modulates angiogenesis

by blocking the CD6-mediated antiangiogenic effect of thrombospondins, THBS1 and THBS2. Acts as a regulator of the vascular endothelial growth factor (VEGF) signaling pathway; inhibits endothelial cell motility by reducing VEGF-induced complex formation between PXN/paxillin and ILK/integrin-linked protein kinase and by promoting inhibition of VEGF-induced tyrosine phosphorylation of focal adhesion kinases and alpha-actinins in endothelial cells. Also plays a role in the regulation of tumor angiogenesis and tumor immune surveillance. Normalizes tumor vessels and promotes antitumor immunity by polarizing tumor-associated macrophages, leading to decreased tumor growth and metastasis. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for HRG / HPRG has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any HRG / HPRG present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for HRG / HPRG is added to each well and incubated. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of HRG / HPRG bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm \pm 2nm. The concentration of HRG / HPRG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Expiration date: Six months at 4°C and twelve months at -20°C.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 10 ng/Vial	4°C
Standard/Sample diluent	30 ml (Ready to use)	4°C
Antibody conjugate concentrate (100X)	1 vial (100 µl)	4°C
Antibody diluent buffer	12 ml (Ready to use)	4°C
HRP-Streptavidin concentrate (100X)	1 vial (100 µl)	4°C
HRP-Streptavidin diluent buffer	12 ml (Ready to use)	4°C
25X Wash Buffer	20 ml	4°C
TMB substrate	10 ml (Ready to use)	4°C (Protect from light)
STOP solution	10 ml (Ready to use)	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C for few minutes before use.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. 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. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

- a) Do not use haemolytic, icteric or lipaemic specimens.
- b) Samples containing sodium azide should not be used in the assay.

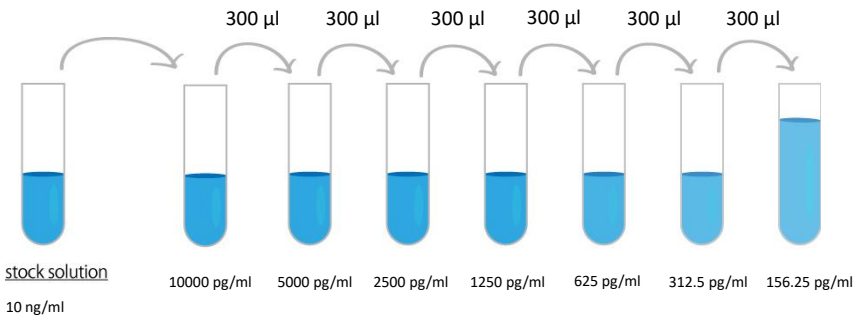
REAGENT PREPARATION

- **1X Wash buffer:** Dilute **25X** Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 20 ml of 25X Wash buffer + 480 ml of distilled water)
The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody conjugate:** It is recommended to prepare this reagent immediately prior to use and use it within 2 hours after preparation.
Dilute 100X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution. (e.g. 10 µl of 100X antibody conjugate concentrate + 990 µl of Antibody diluent buffer)
- **1X HRP-Streptavidin Solution:** It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation.
Dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of HRP-Streptavidin diluent buffer)
- **Sample:** If the initial assay found samples contain HRG / HPRG higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.
(It is recommended to do pre-test to determine the suitable dilution factor).
- **Standards:** Standard solution should be prepared within 2 hours prior to the experiment. Reconstitute the standard with **1 ml Standard/Sample**

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diluent to yield a stock concentration of **10 ng/ml**. Allow the stock standard to sit for at least 10 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 10000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml.

Note: The reconstituted standard solutions are best used within 2 hours. The stock standard solution should be stored at 4°C for up to 12 hours, or aliquot & store at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.



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Dilute HRG / HPRG standard as according to the table below:

Standard	HRG / HPRG Conc. (pg/ml)	μ l of Standard/Sample diluent	μ l of standard
S7	10000 pg/ml	0	1000 (10 ng/ml Stock)
S6	5000 pg/ml	300	300 (S7)
S5	2500 pg/ml	300	300 (S6)
S4	1250 pg/ml	300	300 (S5)
S3	625 pg/ml	300	300 (S4)
S2	312.5 pg/ml	300	300 (S3)
S1	156.25 pg/ml	300	300 (S2)
S0	0	300	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) or 37°C before use. The 1X HRP-Streptavidin Solution and TMB substrate should be prewarm at 37°C few minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard HRG / HPRG detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of HRG / HPRG amount in samples. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 μ l** of standards, samples and zero controls (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for **90 minutes** at **37°C**.
3. Aspirate each well. Complete removal of liquid by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry

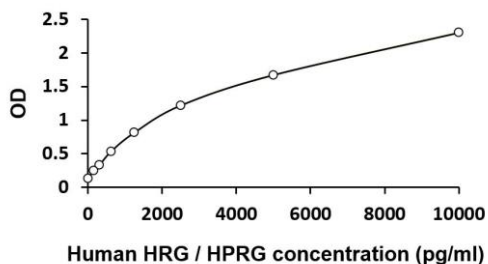
- at any time. Wash step is not necessary in this step.
4. Add **100 µl 1X Antibody conjugate** into each well, gently tap the plate to mix well. Cover wells and incubate for **60 minutes at 37°C**.
 5. Aspirate each well and wash, repeating the process two times for a total three washes. Wash by filling each well with **1X Wash Buffer** (or 0.01M PBS or TBS) (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. Repeat 2 times, total 3 times. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
 6. Add **100 µl of 1X HRP-Streptavidin solution** to each well, gently tap the plate to mix well. Cover wells and incubate for **30 minutes at 37°C**.
 7. Wash as step5, but wash for total 5 times.
 8. Add **90 µl of TMB substrate** to each well. Incubate for **15-30 minutes at 37°C in dark**. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated HRG / HPRG standard solutions; the other wells show no obvious color).
 9. Add **100 µl of Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
 10. Read the OD with a microplate reader at 450nm immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (<https://www.arigobio.com/elisa-analysis>)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human HRG / HPRG ranged from 156 - 10000 pg/ml. The mean MDD was 78 pg/ml.

Specificity

This assay recognizes natural and recombinant Human HRG / HPRG. No significant cross-reactivity or interference with the factors below was observed: There is no detectable cross-reactivity with other relevant proteins.

Intra-assay and Inter-assay precision

The CV values of intra-assay was 4.9% and inter-assay was 7%.