HISTIDINE - RICH GLYCOPROTEIN (HRGP) (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN HRGP CONCENTRATIONS IN SERUM

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

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

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN HRGP/HRG ELISA KIT
Catalog No.	SK00492-01
Lot No.:	
Formulation	96 T
Standard range	62.5 - 4000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Dilution Factor	*20000~40000 (Optimal dilutions should be determined by each laboratory for each application)*
Sample Type	Serum, Plasma
Specificity	Human HRGP
Calibration	Human HRGP recombinant (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C up to 1 month, see page 2 for more information
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Histidine-rich Glycoprotein (HRGP)ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human HRGP from serum in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human HRGP. The capture antibody can bind to the human HRGP in the standard and samples. After washing the plate of any unbound substances, a monoclonal antibody-HRP conjugate against human HRGP is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human HRGP 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.
_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
HRGP Microplate – 96 well microplate precoated with anti-human HRGP monoclonal antibody.	**492-01-01**	**1 plate**
HRGP Standard – 4000 pg/vial of recombinant human HRGP in a buffered protein base with preservative; lyophilized.	**492-01-02**	**2 vials**
Detection Antibody-HRP Conjugate – 110 µL/vial of 100-fold concentrated solution of antibody conjugated to HRP against HRGP.	**492-01-03**	**1 vial**
Positive Control Concentrated – one vial of recombinant human HRGP; lyophilized (optional).	**492-01-04**	**1 vial**
Dilution Buffer - 45 mL of buffered protein based solution with preservative.	**DB02**	**2 bottles**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB02**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL 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 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control and Dilution Buffer should be stored at -20° C. Detection Antibody-HRP Conjugate and TMB Substrate Solution should be stored only at 2 – 8° C (DO NOT FREEZE and PROTECT FROM LIGHT). Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.

- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

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 or plasma samples may need a 20000-fold or 40000-fold dilution. A suggested 100-fold dilution is 5 µL sample + 495 µL Dilution Buffer. Then, to make a final 10000-fold dilution of samples is 5 µL of 100-fold diluted sample + 495 µL Dilution Buffer.

Add 50 µL Dilution Buffer to all sample wells. Then, add 50µL of the final 10000-fold dilution of samples to each sample wells. This final dilution factor is 20000 (20K)-fold diluted.

Add 75 µL Dilution Buffer to all sample wells. Then, add 25 µL of the final 10000-fold dilution of samples to each sample wells. This final dilution factor is 40000 (40K)-fold diluted.

Optimal dilutions should be determined by each laboratory for each application with a pretest. 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.

HRGP Standard - Reconstitute the HRGP standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **4000 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	0.5 ml	4000 pg/ml
# 1	250µl of stock	250µl	2000 pg/ml
# 2	250µl of 1	250µl	1000 pg/ml
# 3	250µl of 2	250µl	500 pg/ml
# 4	250µl of 3	250µl	250 pg/ml
# 5	250µl of 4	250µl	125 pg/ml
# 6	250µl of 5	250µl	62.5 pg/ml

Positive Control Concentrated- Reconstitute the Positive Control with 1 mL of Dilution Buffer (DB02) to make 4-fold concentrated stock solution. Pipette 0.3 mL of Dilution Buffer and transfer 100 µL of 4-fold concentrated stock solution to prepare 1 x working solution.

Detection Antibody-HRP Conjugate - Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.**

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 microplate 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. Add 75 µL Dilution Buffer to all sample wells. Then, add 25 µL of the final 10000-fold dilution of samples to each sample wells. This final dilution factor is 40000 (40K)-fold diluted.
4. Add 100 µl per well of standard dilutions from #6 to #S (reverse order of serial dilution), positive control. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (350 rpm).
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker (350 rpm). **Protect from light.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate Solution to each well. Incubate for 8-12 minutes on microplate shaker at room temperature. **Protect from light**.
9. 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 using a microplate reader set to 450 nm within 3 min.

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 or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted by 20K or 40K, the concentration read from the standard curve must be multiplied by the dilution factor 20K or 40K.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.050)
62.5	0.046
125	0.109
250	0.216
500	0.401
1000	0.795
2000	1.495
4000	2.601

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human HRGP	100
Human Periostin	0
Human ACTR-IIB	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, or positive control to the well. Add 75 µL Dilution Buffer to all sample wells. Then, add 25 µL of the final 10000-fold dilution of samples to each sample wells. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody-HRP working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 8-12 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.