HUMAN GASTRIC INTRINSIC FACTOR (GIF) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SECRETED GIF CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ 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 SECRETED GIF ELISA KIT**
Catalog No.	SK00044-09
Lot No.	
Formulation	96 T
Standard Range	15.6 - 1000 pg/mL
Sensitivity	7 pg/mL
Sample Volume	100 µL
Sample Type	Serum or plasma
Pretreatment	**May require**
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human GIF
Calibration	Human GIF recombinant (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C for 1 month. More information see page 2-3
This kit contains sufficient materials to run 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Secreted GIF ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Secreted GIF from serum and plasma in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Secreted GIF. The capture antibody can bind to the human Secreted GIF in the standard and samples. After washing the plate of any unbound substances, an Antibody-HRP conjugate against human Secreted GIF 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 Secreted GIF 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest.
_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
GIF Microplate - 96 well polystyrene microplate coated with a monoclonal anti-human GIF antibody.	**044-09-01**	**1 plate**
GIF Standard – refer to lot of recombinant human GIF in a buffered protein base with preservative; lyophilized.	**044-09-02**	**1 vial**
Detection Antibody-HRP Conjugate – 105 µL/vial of 100-fold concentrated solution of antibody conjugated to HRP against GIF.	**044-09-03**	**1 vial**
Positive Control – one vial of recombinant human GIF; lyophilized (optional).	**044-09-04**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
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**
Plastic Pouch	**P01**	**1**

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. Antibody-HRP Conjugate and Substrate Solution should be stored only at 2 – 8° C for up to 10 months (**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 (300 – 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.
- Sample Pretreatment Solution: Antibody Eluent Solution (pH 3.0).
- Protein A or Protein G affinity column.

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.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

***** *Due human serum or plasma samples may have circulating anti GIF antibody to interface this immunoassay, human circulating samples may require sample pretreatment and through Protein A or Protein G affinity column.*

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.

GIF Standard - Reconstitute the GIF standard with refer to lot of Dilution Buffer. This reconstitution produces a stock solution of 1000 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 **1000 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	Refer to lot	1000 pg/ml
# 1	250 µl of stock	250 µl	500 pg/ml
# 2	250 µl of 1	250 µl	250 pg/ml
# 3	250 µl of 2	250 µl	125 pg/ml
# 4	250 µl of 3	250 µl	62.5 pg/ml
# 5	250 µl of 4	250 µl	31.25 pg/ml
# 6	250 µl of 5	250 µl	15.6 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

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. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 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.

5. Add 100 µL of 1x Detection Antibody-HRP conjugate working solution to each well. Cover with plate sealer. Incubate for 90 min on microplate shaker at room temperature. **Protect from light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 - 30 minutes on microplate shaker at room temperature. **Protect from light.**
8. 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.
9. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

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 curve fit to more accurately quantify the standard dilutions.

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

TYPICAL DATA

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

Standard (pg/mL)	Average OD450 (Corrected)
Blank	0 (0.093)
15.6	0.042
31.25	0.078
62.5	0.162
125	0.348
250	0.714
500	1.322
1000	2.569

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Secreted GIF	100
Human GIP	0
Human Gastrokine-1 (GKN1)	0

Human GIF recombinant derived from E. Coli may do not be detected by this ELISA kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. 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 90 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 20 - 30 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.