User Guide

Human Ghrelin (Total) ELISA Kit

96-Well Plate

EZGRT-89K
EZGRT-89BK

Intended Use

This kit is used for the non-radioactive quantification of total human ghrelin (both intact and des-octanoyl forms) in serum and plasma. Circulating ghrelin is a multifunctional hormone produced primarily by the stomach. It consists of 28 amino acids and the n-octanoylation of serine3 position in the molecule is necessary for its bioactivity. Originally found as an endogenous ligand for the growth hormone secretagogue receptor in the pituitary gland, it distinguishes itself from the hypothalamic growth hormone-releasing hormone as another potent stimulator for growth hormone secretion. It is also an important orexigenic hormone in the regulation of energy homeostasis. One kit is sufficient to measure 39 unknown samples in duplicate.

This kit is for research use only. Not for use in diagnostic procedures.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- Capture of human ghrelin molecules (both active and des-octanoyl forms) from samples to the wells of a microtiter plate coated with anti-human ghrelin IgG and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titered amount of anchor antibodies.
- Binding of a second biotinylated antibody to the captured molecules
- Washing of unbound materials from samples
- Binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies
- Washing of excess free enzyme conjugates
- Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3’,5,5’-tetramethylbenzidine

The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm–590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured total Human Ghrelin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Human Ghrelin.

Reagents Supplied

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8 °C.

Reagents Supplied	Volume	Quantity	Cat. No.
Microtiter Plate with 2 plate sealers **Note:** Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2–8 °C.	-	1 strip plate 2 sealers	EPRAM
10X HRP Wash Buffer 10X concentrate of 50 mM Tris Buffered Saline containing Tween®-20.	50 mL/bottle	2 bottles	EWB-HRP
Human Ghrelin (Total) Standard	Lyophilized 2 mL	1 bottle	E8089-K
Human Ghrelin (Total) Quality Controls 1 & 2	Lyophilized 0.5 mL	1 vial each	E6089-K
Human Ghrelin (Total) Matrix Solution	1 mL	1 vial	EMTX-GT
Assay Buffer 0.05 M phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.05% Triton™ X-100, 0.08% sodium azide, and 0.1% BSA.	15 mL	1 vial	EABGR
Human Ghrelin (Total) Capture Antibody	3 mL	1vial	E1089-C
Human Ghrelin (Total) Detection Antibody	3 mL	1 vial	E1089-D
Enzyme Solution	12 mL	1 vial	EHRP
Substrate 3,3′,5,5′-tetramethylbenzidine in buffer. Minimize exposure to light.	12 mL	1 vial	ESS-TMB2
Stop Solution 0.3 M HCl (**Caution:** corrosive solution)	12 mL	1 vial	ET-TMB

Storage and Stability

Recommended storage for kit components is 2-8 °C. All components are shipped and stored at 2-8 °C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

Reagent Precautions

Sodium Azide

Sodium azide or Proclin™ has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide and Proclin™ may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Hydrochloric Acid

Hydrochloric acid is corrosive, can cause eye and skin burns. Harmful if swallowed. Causes respiratory and digestive tract burns. Avoid contact with skin and eye. Do not swallow or ingest.

Note: See full labels of hazardous components on next page.

Symbol Definitions

Ingredient	Cat. No.	Full Label	
Human Ghrelin (Total) Capture Antibody	E1089-C		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Human Ghrelin (total) Detection Antibody	E1089-D		**Warning:** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Human Ghrelin (total) Quality Controls 1 & 2	E6089-K	 	**Danger:** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Human Ghrelin (Total) Standard	E8089-K	 	**Danger:** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Assay Buffer	EABGR		**Warning:** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Ingredient	Cat. No.	Full Label	
Stop Solution	ET-TMB		**Warning:** May be corrosive to metals.
10X HRP Wash Buffer Concentrate	EWB-HRP		**Warning:** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
- Buffer and Reagent Reservoirs
- Vortex Mixer
- De-ionized water
- Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm.
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth
- Pefabloc® or AEBSF [4-(2-Aminoethyl)-benzenesulfonyl fluoride], 100 mg/mL aqueous stock solution (store at 2-8 °C, minimize multiple freeze/thaw cycles) is recommended for use in Sample Collection and Storage.
- 5 N HCl, recommended for Sample Collection and Storage.

Sample Collection and Storage

Preparation of Serum Sample and Plasma Samples

The active ghrelin molecule is extremely unstable in serum/plasma and should be rigorously protected during blood sample collection. Ideally all samples should be processed as quickly as possible and kept on ice to retard the breakdown of active ghrelin. For maximum protection, we recommend addition of Pefabloc® or AEBSF and acidification of all samples. Neat samples without such treatment exhibit ~30% (range 20 %-60 %) less total ghrelin content then samples that have been protected. Acidification will result in noticeable protein precipitation but does not affect the assay. However, if the presence of precipitates interferes with the sample pipetting accuracy, the sample should be centrifuged and the supernatant used for assay.

1. To prepare serum, whole blood is directly drawn from Vacutainer® serum tube that contains no anti-coagulant. Immediately add enough AEBSF to a final concentration of 1 mg/mL. Let blood clot at room temperature for 30 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x *g* for 15 minutes at 4 ±2 °C.
3. Transfer serum samples in separate tubes and acidify with HCl to a final concentration of 0.05 N. Aliquot acidified serum in small quantities. Date and identify each sample.
4. Use freshly prepared serum or store samples at –20 ±5 °C for later use. Avoid multiple (> 5) freeze/thaw cycles.
5. To prepare plasma sample, whole blood should be collected into Vacutainer® EDTA-plasma tubes and treated with AEBSF as described for serum, followed by immediate centrifugation. Acidify plasma samples with HCl to a final concentration of 0.05 N. Observe same precautions in the preparation of serum samples.
6. If heparin is to be used as anti-coagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
7. Avoid using samples with gross hemolysis or lipemia.

Reagent Preparation

Human Ghrelin (Total) Standard Preparation

8. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human Ghrelin (Total) Standard with 2 mL of deionized water. Please refer to the analysis sheet for exact concentration. Invert and mix gently until completely in solution.

9. Label five tubes 1, 2, 3, 4, and 5. Add Assay Buffer to each of the five tubes according to the volumes outlined in the chart below. Dilute the reconstituted standard stock according to the chart below. Vortex each tube briefly to ensure complete mixing.

   Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ −20 °C. Avoid multiple freeze/thaw cycles.

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
2 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
1	500 µL	500 µL of reconstituted standard	X/2
2	500 µL	500 µL of Tube 1	X/4
3	500 µL	500 µL of Tube 2	X/8
4	500 µL	500 µL of Tube 3	X/16
5	500 µL	500 µL of Tube 4	X/32

Human Ghrelin (Total) Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Human Ghrelin (Total) Quality Control 1 and Quality Control 2 with 0.5 mL distilled or deionized water and gently invert to ensure complete hydration. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ −20 °C. Avoid further freeze/thaw cycles.

Preparation of Capture and Detection Antibody Mixture

Prior to use, combine the entire contents of Human Ghrelin (Total) Capture Antibody (3 mL) and Human Ghrelin (Total) Detection Antibody (3 mL) at a 1:1 ratio and invert to mix thoroughly.

Human Ghrelin (Total) ELISA Assay Procedure

Warm all reagents to room temperature before setting up the assay.

1. Dilute the 10X concentrated HRP wash buffer 10-fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.

2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8 °C. Assemble the strips in an empty plate holder and fill each well with 300 µL diluted Wash Buffer. Decant wash buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 2 additional times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

3. Add 20 µL Matrix Solution to Blank, Standards and Quality Control wells (refer to Microtiter Plate Arrangement for suggested well orientations).

4. Add 30 µL assay buffer to each of the Blank and sample wells.

5. Add 10 µL assay buffer to each of the Standard and Quality Control wells.

6. Add in duplicate 20 µL Ghrelin Standards in the order of ascending concentrations to the appropriate wells.

7. Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells.

8. Add sequentially 20 µL of the unknown samples in duplicate to the remaining wells.

9. Transfer the Antibody Solution Mixture (1:1 mixture of capture and detection antibody) to a buffer/reagent reservoir and add 50 µL to each well with a multi-channel pipette.

10. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.

11. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.

12. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

13. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 min on the micro-titer plate shaker.

14. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid.

15. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

16. Add 100 µL of Substrate solution to each well, cover plate with sealer and shake in the plate shaker for approximately 5-20 minutes. Blue color should be formed in wells of Ghrelin standards with intensity proportional to increasing concentrations of Ghrelin.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

17. Remove sealer and add 100 µL stop solution (**Caution:** Corrosive solution) and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn into yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there is no air bubbles in any well.

Assay Procedure for Human Ghrelin (Total) ELISA Kit

Well #	Step 1	Step 2	Step 3 Matrix Solution	Step 4-5 Assay Buffer	Step 6-8 Standards/ QCs/Samples	Step 9 Capture/ Detection Antibody	Step 10-12	Step 13 Enzyme Solution	Step 14-15	Step 16 Substrate	Step 16	Step 16 Stop	Step 16
A1, B1	Dilute both bottles of 10X HRP Wash Buffer with 900 mL de-ionized water.	Wash plate 3X with 300 µL diluted HRP Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	20 µL	30 µL	-	50 µL	Seal, Agitate, Incubate 2 hours at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 6X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate for 5-20 minutes at Room Temperature.	100 µL	Read Absorbance at 450 nm and 590 nm.
C1, D1			20 µL	10 µL	20 µL of Tube 5 Std								
E1, F1			20 µL	10 µL	20 µL of Tube 4 Std								
G1, H1			20 µL	10 µL	20 µL of Tube 3 Std								
A2, B2			20 µL	10 µL	20 µL of Tube 2 Std								
C2, D2			20 µL	10 µL	20 µL of Tube 1 Std								
E2, F2			20 µL	10 µL	20 µL of Reconstituted Std								
G2, H2			20 µL	10 µL	20 µL of QC 1								
A3, B3			20 µL	10 µL	20 µL of QC 2								
C3, D3			-	30 µL	20 µL of Sample 1								
E3, F3			-	30 µL	20 µL of Sample 2								
G3, H3, etc.			-	30 µL	20 µL of Sample 3								

Microtiter Plate Arrangement

Human Ghrelin (Total) ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 2 Std.	QC 2	Etc.								
B	Blank	Tube 2 Std.	QC 2	Etc.								
C	Tube 5 Std.	Tube 1 Std.	Sample 1									
D	Tube 5 Std.	Tube 1 Std.	Sample 1									
E	Tube 4 Std.	Reconstituted Standard	Sample 2									
F	Tube 4 Std.	Reconstituted Standard	Sample 2									
G	Tube 3 Std.	QC 1	Sample 3									
H	Tube 3 Std.	QC 1	Sample 3									

Calculations

Graph a reference curve by plotting the absorbance unit of 450 nm, less unit at 590 nm, on the Y-axis against the concentrations of Ghrelin standard on the X-axis. The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function.

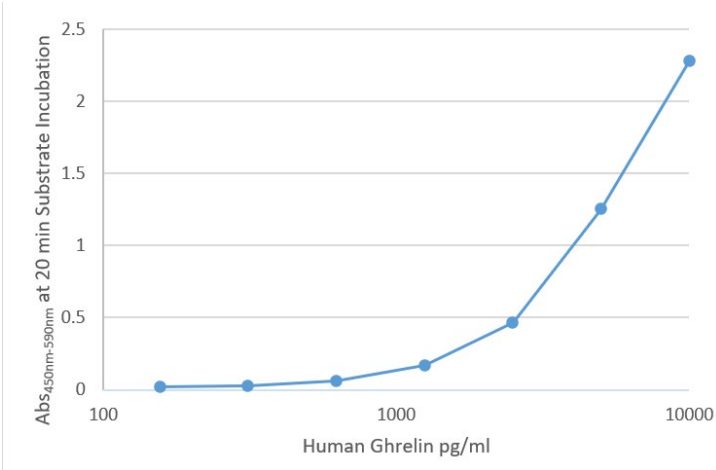
Note: When sample volumes assayed differ from 20 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (for example, if 10 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µL, compensate the volume deficit with matrix solution.

Interpretation

1. The assay will be considered accepted when all Quality Control values fall within the calculated QC range. If any QCs fall outside of the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is > 15% CV, repeat the sample.
3. The theoretical minimal detecting concentration of this assay is 156 pg/mL Total Ghrelin (20 µL sample size).
4. The appropriate range of this assay is 156 pg/mL to 10,000 pg/mL Total Ghrelin (20 µL sample size). Any result greater than 10,000 pg/mL in a 20 µL sample should be diluted using matrix solution and the assay repeated until the results fall within range.

Graph of Typical Reference Curve

Human Ghrelin (Total) ELISA



For demonstration only—Do not use for calculations.

Assay Characteristics

Sensitivity

The lowest level of Total Ghrelin that can be detected by this assay is 100 ng/mL when using a 20 µL sample size.

Specificity

Human Ghrelin (Active)	80%
Des-Octanoyl Human Ghrelin	100%
Canine Ghrelin (Active)	70%
Porcine-Ghrelin (Active)	0%
Motilin Related Peptide (Human, Rat/Mouse)	0%
PYY 3~36 (Human, Mouse, Porcine)	0%
NPY (Human/Rat)	0%
Pancreaetic Polypeptide (Human, Rat)	0%
Human GIP (1-42)	0%
Human GIP (3-42)	0%
Human Insulin	0%
Human Leptin	0%
Human GLP-1	0%
Human C-peptide	0%
Human Amylin	0%
Glucagon	0%
Rat/Mouse Ghrelin (Active)	52%*
Des-Octanoyl Rat/Mouse Ghrelin	54%*

*Purified ghrelin only. This kit should not be used for ghrelin assay in rat/mouse serum or plasma.

Precision

Intra and Inter-Assay Variation

Sample	Mean Total Ghrelin Levels (pg/mL) Mean, n =6	Intra-Assay % CV	Inter-Assay % CV
#1, Serum	384.6	1.26	7.81
#2, Serum	904.5	0.90	6.28
#3, Serum	1,522.4	0.99	6.18
#4, Plasma	272.1	1.76	7.74
#5, Plasma	868.4	1.11	5.18
#6, Plasma	1,346.7	1.91	6.53

The assay variations of Human Ghrelin (Total) ELISA kits were studied on three fasting human serum and plasma samples with varying concentrations of endogenous ghrelin. Intra-assay variations were calculated from results of six duplicate determinations in one assay. Inter-assay variations were calculated from results of six separate assays with duplicate samples in each assay.

Spike Recovery of Total Human Ghrelin in Assay Samples

Sample I.D.	Ghrelin Spiked (pg/mL)		Serum Ghrelin		Plasma Ghrelin	
			pg/mL	Recovery Rate	pg/mL	Recovery Rate
1	Intact Ghrelin	0 (Basal)	101	-	115	-
		250	298	79%	312	79%
		1,000	868	77%	877	76%
		2,000	1,528	71%	1,571	73%
2		0 (Basal)	397	-	298	-
		250	662	103%	553	102%
		1,000	1,536	100%	1,291	99%
		2,000	2,677	99%	2,196	95%
3		0 (Basal)	842	-	712	-
		250	1,072	92%	913	81%
		1,000	1,708	87%	1,561	85%
		2,000	2,564	86%	2,408	85%
Mean ±S.D. (n = 3)		250	-	91.3 ±12.0%	-	87.3 ±12.7%
		1,000	-	88.0 ±11.5%	-	86.7 ±11.6%
		2,000	-	85.3 ±14.0%	-	84.3 ±11.0%
1	Des-Octanoyl Ghrelin	0 (Basal)	124	-	85	-
		250	347	89%	365	112%
		1,000	1,051	93%	1,140	106%
		2,000	1,922	90%	2,088	100%
2		0 (Basal)	397	-	312	-
		250	662	106%	609	119%
		1,000	1,536	114%	1,600	129%
		2,000	2,677	114%	2,887	129%
3		0 (Basal)	886	-	703	-
		250	1,155	108%	992	116%
		1,000	2,031	115%	1,910	121%
		2,000	3,086	110%	3,057	118%
Mean ±S.D. (n = 3)		250	-	101.0 ±10.4%	-	115.7 ±3.50%
		1,000	-	107.3 ±12.4%	-	118.7 ±11.7%
		2,000	-	104.7 ±12.9%	-	112.3 ±15.0%

Varying amounts of active or des-octanoyl human ghrelin were added to 3 post-prandial human serum and plasma samples and the ghrelin content of each sample was assayed by Human Ghrelin (Total) ELISA. The recovery rate = [(Observed ghrelin concentration after spike – Basal ghrelin level) / spiked ghrelin concentration] x 100%.

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

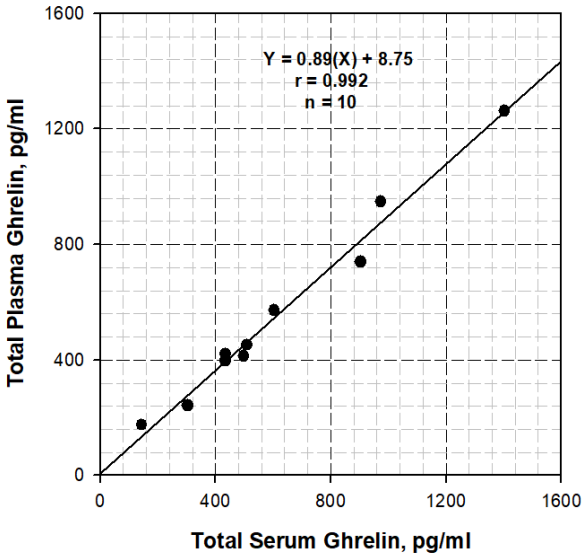
Linearity of Sample Dilution

Sample I.D.	Volume Assayed	Serum Ghrelin		Plasma Ghrelin	
		pg/mL	% of Expected	pg/mL	% of Expected
1	20 µL	719	100%	598	100%
	15 µL	511	95%	431	96%
	10 µL	352	98%	294	98%
	5 µL	192	107%	176	118%
2	20 µL	1,100	100%	1,060	100%
	15 µL	796	96%	723	91%
	10 µL	520	94%	484	91%
	5 µL	276	100%	263	99%
3	20 µL	421	100%	369	100%
	15 µL	300	95%	269	97%
	10 µL	200	95%	200	108%
	5 µL	113	107%	123	133%
Mean ± S.D. (n = 3)	20 µL	-	100%	-	100%
	15 µL	-	95.3 ±0.6%	-	94.7 ±3.2%
	10 µL	-	95.7 ±2.1%	-	99.0 ±8.5%
	5 µL	-	104.7 ±4.0%	-	116.7 ±17%

Fasting serum and plasma samples from 3 individuals were assayed at 20, 15, 10 and 5 µL each for total ghrelin by ELISA. Measured ghrelin levels are corrected for various dilution factors and then divided by levels found at 20 µL sample size to obtain the % of expected values.

Normal Range of Total Ghrelin in Human Blood

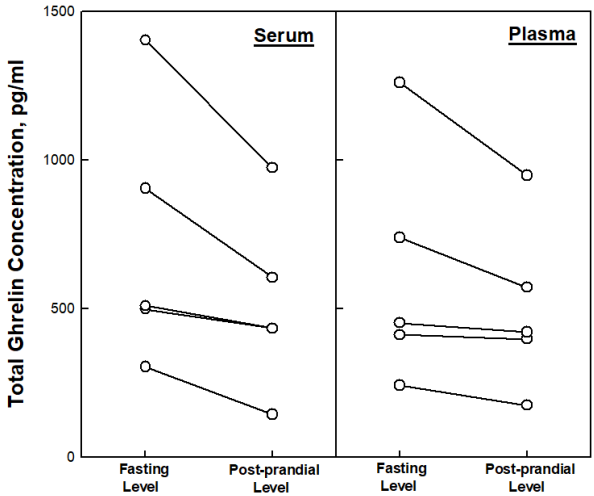
Correlation Between Serum and Plasma Concentrations



Fasting and post-prandial serum and plasma sample from 5 individuals were assayed for total ghrelin by ELISA and the paired results were analyzed by linear regression analysis.

Post-Prandial Attenuation of Total Ghrelin in Blood

Post-Meal Attenuation of Total Ghrelin Level in Blood

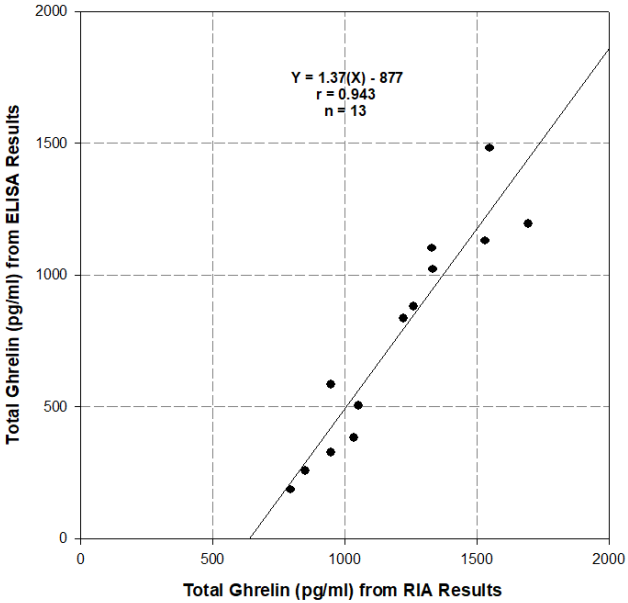


Fasting and 1-hour postprandial serum and plasma from 5 individuals were assayed for total ghrelin using ELISA.

Correlation Graph

RIA GHRT-89HK vs. ELISA EZGRT-89K

Total Human Ghrelin Immunoassays: Correlation Between RIA and ELISA



13 Fasting human serum samples collected with AEBSF & HCl treatment were assayed for total ghrelin level by RIA (GHRT-89HK) and ELISA (EZGRT-89K). Paired results were analyzed using linear regression analysis.

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- High signal in background or blank wells could be due to:
    - cross well contamination by standard solution or sample
    - inadequate washing of wells with HRP Wash Buffer
    - overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Cat. No.
Human Ghrelin (Total) Microtiter Plate	EPRAM
10X HRP Wash Buffer Concentrate	EWB-HRP
Human Ghrelin (Total) Standard	E8089-K
Human Ghrelin (Total) Quality Controls 1 and 2	E6089-K
Matrix Solution	EMTX-GT
Assay Buffer	EABGR
Human Ghrelin (Total) ELISA Capture Antibody	E1089-C
Human Ghrelin (Total) ELISA Detection Antibody	E1089-D
Enzyme Solution	EHRP
Substrate Solution	ESS-TMB2
Stop Solution	ET-TMB
10-pack of Human Ghrelin (Total) ELISA Kits	EZGRT-89BK

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