DRG® Human Ghrelin (total) ELISA (EIA-4709)

Revised 6 Aug. 2009 (Vers. 2.0) RUO

Please use only the valid version of the package insert provided with the kit.

1 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 purpose only.

2 PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based on:
1) capture of human ghrelin molecules (both active and des-octanoyl forms) in the sample by 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,
2) and the simultaneous binding of a second biotinylated antibody to ghrelin,
3) wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies,
4) wash away of free enzyme, and
5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetra-methylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured total human ghrelin in the unknown sample, the concentration of total ghrelin can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human ghrelin.

3 REAGENTS SUPPLIED

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

1. **Microtiter Plate**
   Coated with pre-titered anchor antibodies.
   Quantity: 1 Strip Plate
   Preparation: Ready to use.
   Note: Unused strips should be resealed in the foil pouch with the dessicant provided and stored at 2-8 °C.
2. **Adhesive Plate Sealer**
   Quantity: 2 sheets
   Preparation: Ready to use.
3. **10X HRP Wash Buffer Concentrate**
   10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.
   Quantity: 2 bottles containing 50 mL each
   Preparation: Dilute 1:10 with distilled or de-ionized water.

4. **Human Ghrelin (Total) Standard**
   Human Ghrelin (total) reference standard, 5 ng/ml, lyophilized
   Quantity: 1 bottle, 5 ng/ml after reconstitution with appropriate amount of water
   Preparation: Hydrate thoroughly in distilled or de-ionized water immediately before use. Please refer to the analysis sheet for exact amount of water to be used since it will be lot dependent.
   After hydration dilute with Assay Buffer according to § 8.1.

5. **Human Ghrelin (Total) Quality Controls 1 and 2**
   One vial each, lyophilized, containing human ghrelin (total) at two different levels.
   Quantity: 0.5 mL/vial upon hydration
   Preparation: Reconstitute each vial with 0.5 mL de-ionized water immediately before use.
   Aliquot unused portion in smaller quantity and freeze at -20°C for later use. Avoid further freeze and thaw.

6. **Human Ghrelin (Total) Matrix**
   Processed serum matrix containing 0.08% Sodium Azide
   Quantity: 1 mL/vial
   Preparation: Ready to use.

7. **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.
   Quantity: 15 mL/vial
   Preparation: Ready to use.

8. **Human Ghrelin (Total) Capture Antibody**
   Pre-titered capture antibody solution in buffer
   Quantity: 3 mL/vial
   Preparation: Mix thoroughly with Human Ghrelin (Total) Detection Antibody before use according to § 8.3

9. **Human Ghrelin (Total) Detection Antibody**
   Pre-titered detection antibody solution in buffer
   Quantity: 3 mL/vial
   Preparation: Mix thoroughly with Human Ghrelin (Total) Capture Antibody before use according to § 8.3

10. **Enzyme Solution**
    Pre-titered streptavidin-horseradish peroxidase conjugate in buffer.
    Quantity: 12 mL/vial
    Preparation: Ready to use

11. **Substrate**
    3, 3',5,5'-tetramethylbenzidine in buffer.
    Quantity: 12 mL/vial
    Preparation: Ready to use. Minimize the exposure to light.

12. **Stop Solution**
    0.3 M HCl; Caution: Corrosive Solution
    Quantity: 12 mL/vial
    Preparation: Ready to use.

4 STORAGE AND STABILITY

All components of the kit should be stored at 4°C.

Prepare and use standard/QC solutions within a day after reconstitution and aliquot in smaller quantity and store at ≤ -20°C for later use, if necessary. Avoid further freeze/thaw cycles.

Refer to expiration dates on all reagents prior to use.

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Do not mix reagents from different kits unless they have the same lot numbers.

5 REAGENT PRECAUTIONS

1. Sodium Azide

Sodium azide has been added to certain reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, sodium azide may react with lead and copper pluming to form explosive metal azides. On disposal, flush with large volume of water to prevent azide build up.

2. 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.

6 MATERIALS REQUIRED BUT NOT PROVIDED

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

7 SAMPLE COLLECTION AND STORAGE

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 into a 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.

8 REAGENT PREPARATION

8.1 Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human Ghrelin (Total) Standard with the amount of distilled or deionized water specified in the data sheet supplied with this kit to give a final concentration of 5 ng/ml (or 5000 pg/mL) of Total Ghrelin Standard.
   Invert and mix gently until completely in solution.
2. Label five tubes with the additional concentrations of standards to be prepared:
   100 pg/mL, 200 pg/mL, 500 pg/mL, 1000 pg/mL, and 2000 pg/mL.
   Add Assay Buffer to each of the five tubes according to the volumes outlined in the chart below. Dilute the reconstituted 5 ng/ml 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 standard should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Concentration of Standards	Volume of 5 ng/ml Stock to Add	Volume of Assay Buffer to Add
100 pg/mL	0.020 mL	0.980 mL
200 pg/mL	0.040 mL	0.960 mL
500 pg/mL	0.100 mL	0.900 mL
1,000 pg/mL	0.200 mL	0.800 mL
2,000 pg/mL	0.400 mL	0.600 mL
5,000 pg/mL	----	----

8.2 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.

8.3 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.

9 ASSAY PROCEDURE

Pre-warm all reagents to room temperature immediately 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 § 10 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.
    Read absorbance at 450 nm and 590nm in a plate reader within 5 minutes and ensure that there is no air bubbles in any well.

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Assay Procedure for Human Ghrelin (Total) ELISA

	Well #	Step 1	Step 2	Step 3	Step 4-5	Step 6-8	Step 9	Step 10-12	Step 13	Step 14-15	Step 16	
		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	Matrix Solution	Assay Buffer	Standards/QCs/ Samples	Capture/ Detection Ab. Mixture	Seal, Agitate, Incubate 2 hours at Room Temperature. Wash 3X with 300 µl Wash Buffer.	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 6X with 300 µl Wash Buffer.	Substrate	Stop Solution
	A1, B1			20 µl	30 µl	--	50 µl		100 µl		100 µl	100 µl
	C1, D1			20 µl	10 µl	20 µl of 100 pg/mL Standard						
	E1, F1			20 µl	10 µl	20 µl of 200 pg/mL Standard						
	G1, H1			20 µl	10 µl	20 µl of 500 pg/mL Standard						
	A2, B2			20 µl	10 µl	20 µl of 1000 pg/mL Standard						
	C2, D2			20 µl	10 µl	20 µl of 2000 pg/mL Standard						
	E2, F2			20 µl	10 µl	20 µl of 5000 pg/mL Standard						
	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						

Read Absorbance at 450 nm and 590 nm.

10 MICROTITER PLATE ARRANGEMENT

Human Ghrelin (Total) ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	1000 pg/mL	QC2	Etc.								
B	Blank	1000 pg/mL	QC2	Etc.								
C	100 pg/mL	2000 pg/mL	Sample 1									
D	100 pg/mL	2000 pg/mL	Sample 1									
E	200 pg/mL	5000 pg/mL	Sample 2									
F	200 pg/mL	5000 pg/mL	Sample 2									
G	500 pg/mL	QC1	Sample 3									
H	500 pg/mL	QC1	Sample 3									

11 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 (e.g., 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.

12 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 100 pg/mL Total Ghrelin (20 µL sample size).
4. The appropriate range of this assay is 100 pg/mL to 5,000 pg/mL Total Ghrelin (20 µL sample size).
   Any result greater than 5,000 pg/mL in a 20 µL sample should be diluted using matrix solution and the assay repeated until the results fall within range.

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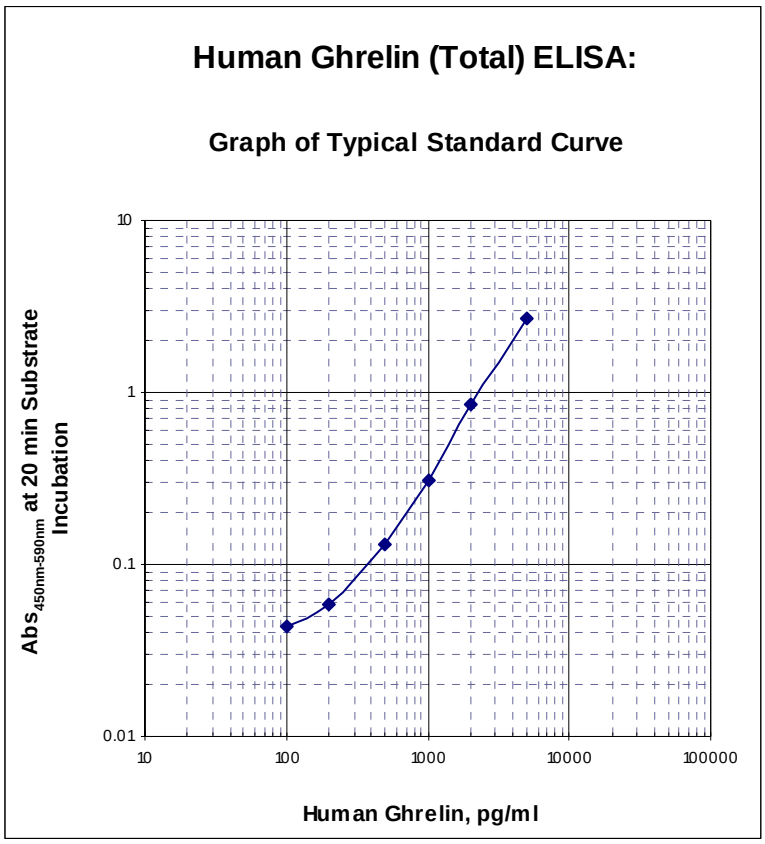
13 GRAPH OF TYPICAL REFERENCE CURVE



For Demonstration Only – Do not use for calculations

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14 ASSAY CHARACTERISTICS

14.1 Sensitivity

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

14.2 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%
Pancreatic 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.

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14.3 Precision

Intra and Inter-Assay Variations

Sample	Total Ghrelin (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.

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14.4 Spike Recovery Rate 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	--
		100	347	89%	365	1112%
		1,000	1,051	93%	1,140	106%
		2,000	1,922	90%	2,088	100%
2		0 (Basal)	397	--	312	--
		100	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	--
		100	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%.

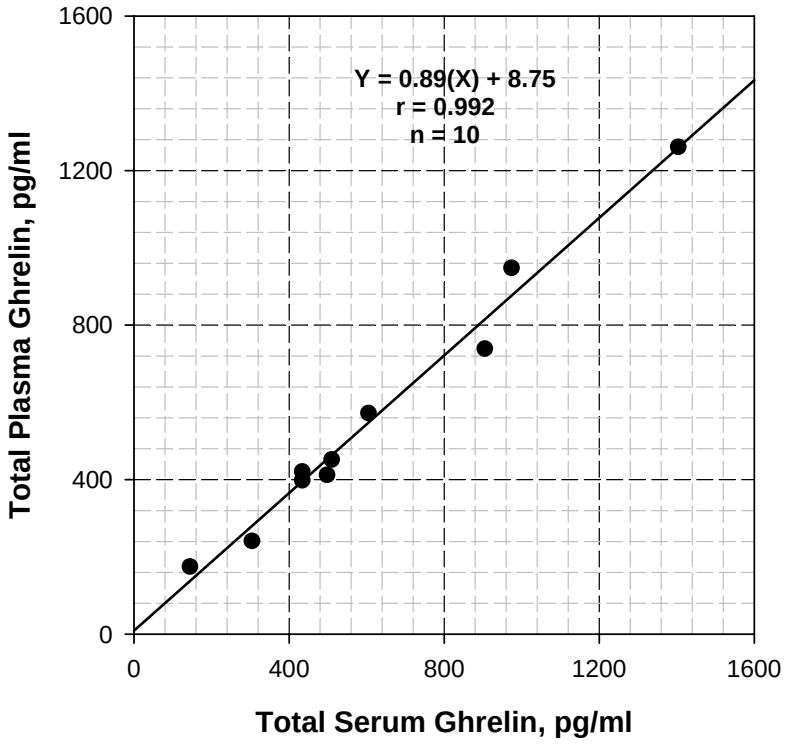
14.5 Linearity of Sample Dilution

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.

15 NORMAL RANGE OF TOTAL GHRELIN LEVELS IN HUMAN BLOOD

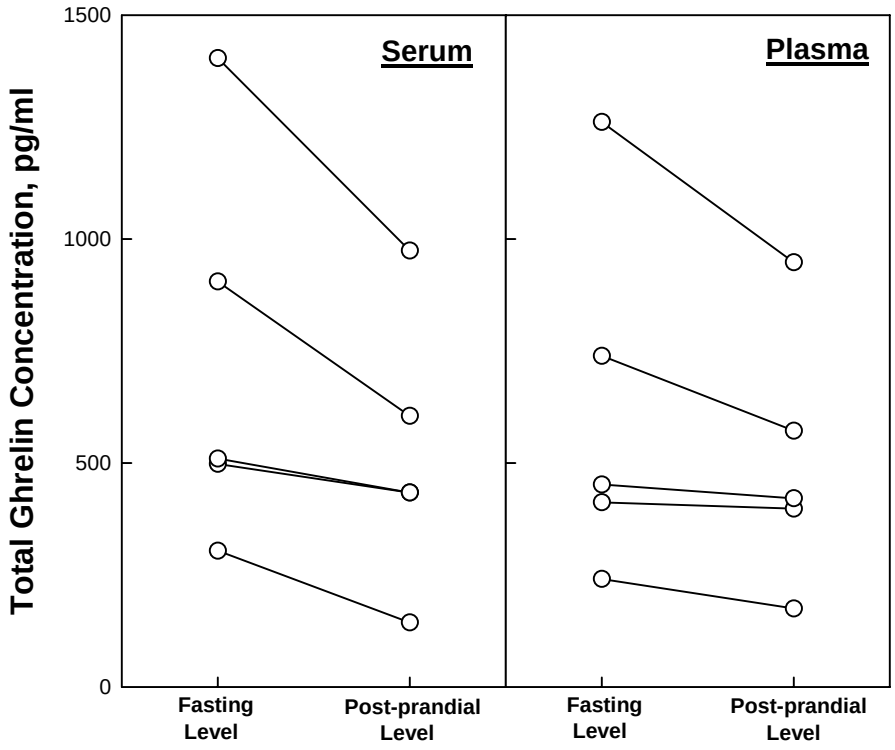
Human Ghrelin (Total) ELISA: Correlation Between Serum and Plasma Concentrations



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

16 POST-PRANDIAL ATTENUATION OF TOTAL GHRELIN IN BLOOD

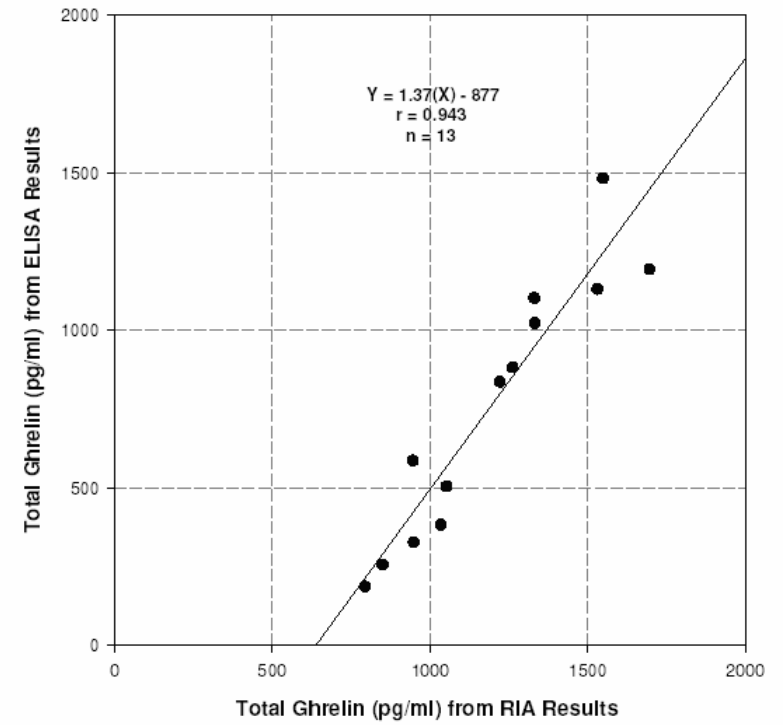
Post-meal Attenuation of Total Ghrelin Level in Blood



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

17 CORRELATION

Total Human Ghrelin Immunoassays: Correlation Between RIA and ELISA



13 Fasting human serum samples collected with AEBSF & HCl treatment are assayed for total ghrelin level by DRG RIA-3967 and ELISA EIA-4709 Paired results are analyzed by linear regression analysis.

18 QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert.

19 TROUBLESHOOTING GUIDE

1. 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.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. 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.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. 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.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.

8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with HRP Wash Buffer or 3) overexposure to light after substrate has been added.

20 ORDERING INFORMATION

Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals. All products are intended for in vitro use only.

Material Safety Data Sheets (MSDS)

Material safety data sheets for the product may be ordered by fax or phone.

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

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