User Guide

Rat/Mouse Ghrelin (Total) ELISA Kit

96-Well Plate

EZRGRT-91K

Intended Use

This kit is used for the non-radioactive quantification of total Rat/Mouse 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 Rat/Mouse ghrelin molecules (both active and des-octanoyl forms) from samples to the wells of a microtiter plate coated with anti-Rat/Mouse ghrelin IgG
- 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, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured total Rat/Mouse 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 Rat/Mouse 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	EP91
10X HRP Wash Buffer	50 mL/bottle	2 bottles	EWB-HRP
Rat/Mouse Ghrelin (Total) Standard	2 mL upon hydration Lyophilized	1 vial	E8091-K
Rat/Mouse Ghrelin (Total) Quality Controls 1 & 2	0.5 mL upon hydration Lyophilized	1 vial	E6091-K
Matrix Solution	1 mL	1 vial	EMTX-GA
Assay Buffer	15 mL	1 vial	EABGR
Rat/Mouse Ghrelin (Total) Detection Antibody	6 mL	1 vial	E1091
Enzyme Solution	12 mL	1 vial	EHRP
Substrate **Note:** Minimize exposure to light.	12 mL	1 vial	ESS-TMB3
Stop Solution (**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	
Rat/Mouse Ghrelin (total) Detection Antibody	E1091		**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.
Rat/Mouse Ghrelin (total) Quality Controls 1 & 2	E6091-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.
Rat/Mouse Ghrelin (Total) Standard	E8091-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.
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 −20 °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

Ghrelin molecules, especially acylated form, are extremely unstable in rat or mouse 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 ghrelin. For maximum protection, we recommend addition of Pefabloc® or AEBSF and acidification of all samples. 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 centrifuge tube that contains no anti-coagulant. Immediately add enough Pefabloc® or 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 a centrifuge tube containing enough K_3EDTA to achieve a final concentration of 1.735 mg/mL and treated with Pefabloc® or 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

Rat/Mouse Ghrelin (Total) Standard Preparation

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

2. Label six tubes 1, 2, 3, 4, 5, and 6. Add Assay Buffer to each of the six 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 (ng/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
6	500 µL	500 µL of Tube 5	X/64

Rat/Mouse Ghrelin (Total) Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Rat/Mouse Ghrelin (Total) Quality Control 1 and Quality Control 2 with 0.5 mL distilled or de-ionized 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.

Rat/Mouse 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. Add 50 µL of Detection Antibody 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 15 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 Rat/Mouse Ghrelin (Total) ELISA Kit

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		
			Matrix Solution	Assay Buffer	Standards/ QCs/Samples	Detection Antibody		Enzyme Solution		Substrate		Stop
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 on a plate shaker. Wash 6X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate for 15 minutes at Room Temperature.	100 µL
C1, D1			20 µL	10 µL	20 µL of Tube 6 Std							
E1, F1			20 µL	10 µL	20 µL of Tube 5 Std							
G1, H1			20 µL	10 µL	20 µL of Tube 4 Std							
A2, B2			20 µL	10 µL	20 µL of Tube 3 Std							
C2, D2			20 µL	10 µL	20 µL of Tube 2 Std							
E2, F2			20 µL	10 µL	20 µL of Tube 1 Std							
G2, H2			20 µL	10 µL	20 µL of Reconstituted Std							
A3, B3			20 µL	10 µL	20 µL of QC 1							
C3, D3			20 µL	10 µL	20 µL of QC 2							
E3, F3			-	30 µL	20 µL of Sample 1							
G3, H3, etc.			-	30 µL	20 µL of Sample 2							

Read Absorbance at 450 nm and 590 nm.

Microtiter Plate Arrangement

Rat/Mouse Ghrelin (Total) ELISA

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

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.

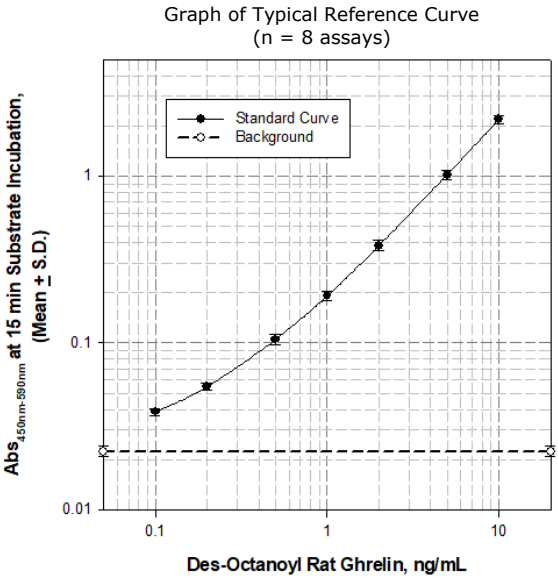
Note: When sample volumes assayed differ from 20 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (for example, if 10 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µL, compensate the volume 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 0.04 ng/mL Total Ghrelin (20 µL sample size).
4. The appropriate range of this assay is 0.04 ng/mL to 10 ng/mL Total Ghrelin (20 µL sample size). Any result greater than 10 ng/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

Rat/Mouse 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 0.04 ng/mL when using a 20 µL sample size.

Specificity

Rat/Mouse Ghrelin (Active)	85%
Des-Octanoyl Rat/Mouse Ghrelin	100%
Human Ghrelin (Active)	115%
Des-Octanoyl Human Ghrelin	249%
Canine-Ghrelin (Active)	71%
Porcine-Ghrelin (Active)	48%
PYY 3-36 (Human, Porcinne), 25 ng/mL	0%
NPY (Human/Rat) 50 nM	0%
Human Pancreaetic Polypeptide, 1 µg/mL	0%
Rat Pancreatic Polypeptide, 0.5 nM	0%
Human GIP (1-42), 1 µg/mL	0%
Rat Leptin, 1 µg/mL	0%
Mouse Leptin, 2 µg/mL	0%

Precision

Intra and Inter-Assay Variation

Sample	Mean Total Ghrelin Levels (ng/mL)	Intra-Assay % CV	Inter-Assay % CV
Rat Serum 1	0.78	0.69	3.34
Rat Serum 2	2.25	0.91	1.76
Rat Serum 3	4.67	0.85	3.31
Mouse Serum 1	0.68	0.82	4.46
Mouse Serum 2	2.35	1.29	3.45
Mouse Serum 3	5.67	1.27	3.52
Rat Plasma 1	2.02	1.67	3.24
Rat Plasma 2	2.90	1.11	1.99
Rat Plasma 3	4.48	1.16	2.43
Mouse Plasma 1	1.84	1.56	2.90
Mouse Plasma 2	3.13	1.07	2.92
Mouse Plasma 3	4.89	1.07	2.81

Serum or plasma samples from rats and mice are pooled and treated with AEBSF and HCl, then divided into 3 aliquots each. Various amounts of des-octanoyl rat ghrelin are added to the aliquots to create low, intermediate and high levels of ghrelin samples for precision tests. 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 Rat Ghrelin in Assay Samples

Sample	I.D.	Basal Total Ghrelin (ng/mL)	% Recovery of Spiked Analytes: Des-Oct Rat Ghrelin Spiked + 0.5 ng/mL	Des-Oct Rat Ghrelin Spiked + 2 ng/mL	Des-Oct Rat Ghrelin Spiked + 5 ng/mL	Acylated Rat Ghrelin Spiked + 0.5 ng/mL	Acylated Rat Ghrelin Spiked + 2 ng/mL	Acylated Rat Ghrelin Spiked + 5 ng/mL
Rat Serum	49455	1.34	92.0	100.0	102.8	71.1	91.5	94.3
	49458	1.70	92.0	101.5	102.4	89.5	94.9	95.1
	49457	1.94	96.0	104.5	104.8	79.0	96.9	99.1
	49456	2.25	94.0	98.0	104.0	79.0	96.2	98.0
	49459	2.53	96.0	102.5	102.2	79.0	96.3	97.2
Mean ± S.D.			92.0 ±4.9%	101.3 ±2.5%	103.2 ±1.1%	79.5 ±6.5%	95.2 ±2.2%	96.7 ±2.0%
Rat Plasma	49475	2.05	94.0	103.5	108.2	76.3	97.3	102.1
	49469	2.46	118.0	109.5	108.2	110.5	104.1	105.0
	49474	2.58	94.0	100.5	104.6	79.0	86.5	84.2
	49473	2.74	96.0	107.5	109.8	97.4	100.1	107.7
	49472	3.19	88.0	100.5	105.4	65.8	92.6	101.6
Mean ± S.D.			98.0 ±11.6%	104.3 ±4.1%	107.2 ±2.2%	85.8 ±17.9%	97.4 ±4.8%	101.8 ±5.6%
Mouse Serum	47950	1.26	102.0	97.0	99.2	102.7	101.1	98.4
	47945	0.86	112.0	111.5	110.4	118.9	113.5	111.4
	47948	1.58	88.0	82.0	82.2	100.0	87.3	82.0
	47951	1.94	106.0	112.5	111.0	97.3	107.6	109.9
	47952	1.13	106.0	107.0	107.0	97.3	101.8	103.9
Mean ± S.D.			102.8 ±9.0%	102.0± 12.8%	102.0± 12.0%	103.2 ±9.0%	102.3 ±9.7%	101.1 ±11.9%
Mouse Plasma	47968	0.68	102.0	99.5	99.6	84.5	96.8	96.9
	47962	1.03	102.0	102.0	103.2	95.8	97.5	100.0
	47967	1.01	96.0	93.0	95.1	84.5	91.8	93.0
	47964	1.50	88.0	84.5	87.8	93.0	91.0	89.9
	47966	2.52	88.0	95.5	100.0	81.7	96.1	97.8
Mean ± S.D.			95.2 ±7.0%	94.9 ±6.8%	97.2 ±5.9%	87.9 ±6.1%	94.6 ±3.0%	95.6 ±4.1%

Varying amounts des-octanoyl or acylated rat ghrelin were added to 5 rat/mouse serum and plasma samples and the ghrelin content of each sample was assayed by Rat/Mouse Ghrelin (Total) ELISA. The recovery rate = [(Observed ghrelin concentration after spike – Basal ghrelin level) / spiked ghrelin concentration] x 100%. Recovery rate of spiked acylated ghrelin is calculated based on the amount of spiked results in assay buffer with matrix solution.

Linearity of Sample Dilution

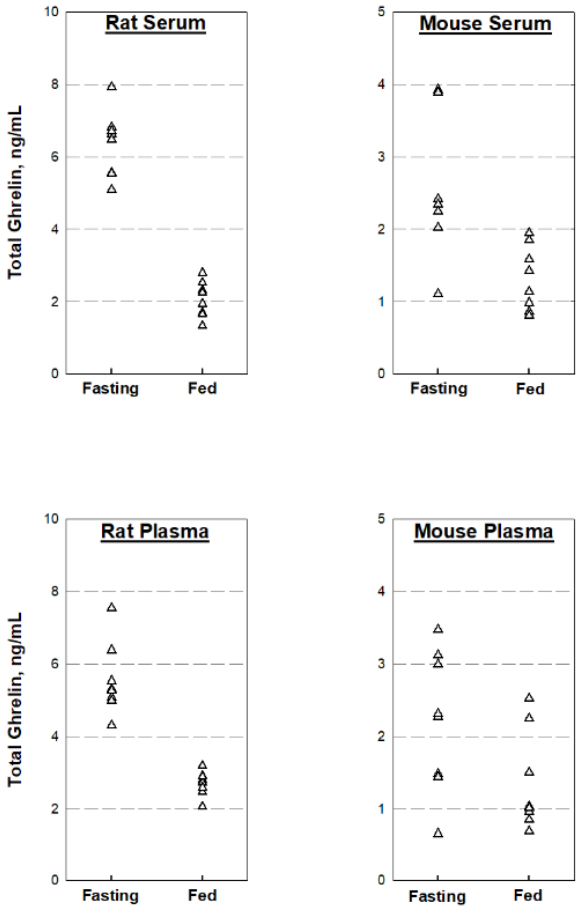
		Total Ghrelin in Sample Volume Assayed and the Dilution Responses						
		20 µL	15 µL		10 µL		5 µL	
Sample	I.D.	ng/mL	ng/mL	%Expect.	ng/mL	%Expect.	ng/mL	%Expect.
Rat Serum	49445	6.62	4.52	91.0	3.03	91.5	1.43	86.4
	49446	6.47	4.95	102.0	3.25	100.5	1.64	101.4
	49447	6.80	5.03	98.6	3.45	101.5	2.11	124.1
	49448	7.91	5.92	99.8	3.98	100.6	1.99	100.6
	49449	6.69	5.09	101.4	3.34	99.9	1.69	101.1
% Expected Mean ±S.D.		100%	98.6 ±4.4%		98.8 ±4.1%		102.7 ±13.5%	
Rat Plasma	49461	6.37	4.74	99.2	3.10	98.1	1.55	98.1
	49462	5.52	4.14	100.0	2.68	97.1	1.33	96.4
	49463	5.29	3.86	97.3	2.56	96.8	1.27	96.0
	49464	5.07	3.70	97.3	2.52	99.4	1.24	97.8
	49465	7.54	5.59	98.9	3.67	97.4	1.84	97.6
% Expected Mean ±S.D.		100%	98.5 ±1.2%		97.8 ±1.0%		97.2 ±0.9%	
Mouse Serum	47980	2.41	1.96	108.4	1.63	135.3	1.32	219.1
	47981	2.02	1.46	96.4	1.00	99.0	0.53	105.0
	47982	2.34	1.72	98.0	1.21	103.4	0.68	116.2
	47983	2.24	1.64	97.6	1.15	102.7	0.63	112.5
	47950	1.57	1.16	98.5	0.80	101.9	0.40	101.9
% Expected Mean ±S.D. (N = 4)		100%	97.6 ±0.9%		101.8 ±1.9%		108.9 ±6.6%	
Mouse Plasma	47953	2.99	2.42	107.9	1.68	112.4	0.88	117.7
	47954	2.27	1.77	104.0	1.20	105.7	0.63	111.0
	47955	3.47	2.32	89.2	1.87	107.8	0.92	106.1
	47958	3.26	2.44	99.8	1.74	106.8	0.89	109.2
	47959	1.48	1.09	98.2	0.71	96.0	0.34	91.9
% Expected Mean ±S.D.		100%	99.8 ±7.0%		105.7 ±6.0%		107.2 ±9.5%	

*Mouse Serum 47980 is extensively hemolyzed and not included in the statistics.

Fasting serum and plasma samples from rats and mice 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 and Post-Prandial Attenuation of Total Ghrelin in Rat/Mouse Blood

Effect of Fasting on Serum/Plasma Ghrelin (Total) Levels

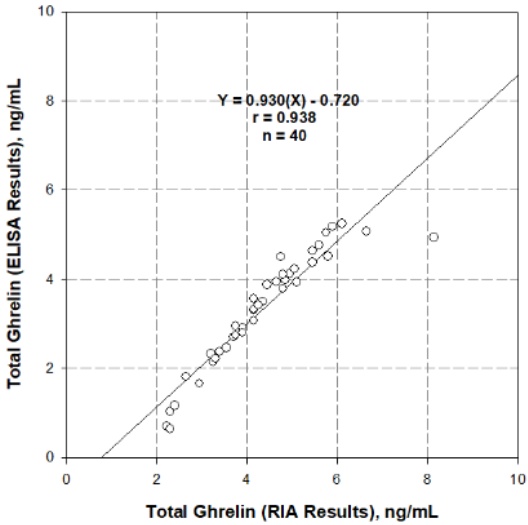


Each group contains 8 animals, either fed *ad lib* or 24-hour fasted before blood collection. All blood samples are treated immediately with 1 mg/mL AEBSF and processed for serum/plasma isolation. Resulting serum/plasma samples are acidified to 0.05 N HCl and stored at −20 °C before ELISA assay.

Correlation Graph

Correlation of Rat/Mouse Ghrelin (Total) Assays Results
RIA vs. ELISA

Serum/plasma samples from rats and mice are pooled separately and treated with



AEBSF and HCl, then spiked with des0octanoyl rat ghrelin to 10 different levels. 20 µL from each sample was assayed for total ghrelin by RIA (Cat. No. GHRT-89K) and ELISA (EZRGRT-91K). Paired results are analyzed by 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, or
    - inadequate washing of wells with Wash Buffer, or
    - overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Cat. No.
Rat/Mouse Ghrelin (Total) Microtiter Plate	EP91
10X HRP Wash Buffer Concentrate	EWB-HRP
Rat/Mouse Ghrelin (Total) Standard	E8091-K
Rat/Mouse Ghrelin (Total) Quality Controls 1 and 2	E6091-K
Matrix Solution	EMTX-GA
Assay Buffer	EABGR
Rat/Mouse Ghrelin (Total) ELISA Detection Antibody	E1091
Enzyme Solution	EHRP
Substrate Solution	ESS-TMB3
Stop Solution	ET-TMB

Notice

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