BioVendor Group

ENG

Instructions for Use:

MOUSE/RAT UNACYLATED GHRELIN EXPRESS ELISA

Catalogue number:

RA394063400R

For research use only.

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.005.A	ENG.006.A
Chapter 12.7. updated.	

1. MOUSE/RAT GHRELIN UNACYLATED EXPRESS ELISA

96 wells
Storage: -20°C
Expiry date: stated on the package

This kit contains:

REAGENTS (Store at 2-8°C)	COLOUR CODE	Quantity	Form
Antibody Coated Microtiter Strips	Blister with zip	1	
Conjugate Solution (Human, rat tracer express)	Green	1	lyophilized
Mouse/Rat Unacylated Ghrelin Standard	Blue with red septum	2	lyophilized
Quality Control	Green with red septum	2	lyophilized
Dilution Buffer (EIA buffer)	Blue	1	lyophilized
Wash Solution Conc. (400x)	Silver	1	liquid
Substrate Solution (Ellman´s reagent)	Black with red septum	2	lyophilized
Tween 20	Transparent	1	liquid
Cover Sheet		1	

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 36 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of Standard, one of Quality Control and one of Substrate Solution.

2. PRECAUTION FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only.
- Not for diagnostic use.
- Do not pipet liquids by mouth.
- Do not use kit components beyond the expiration date.
- Do not eat, drink or smoke in area in which kit reagents are handled.
- Avoid splashing.

The total amount of reagents contains less than 100 µg of sodium azide. Flush the drains thoroughly to prevent the production of explosive metal azides.

Wearing gloves, laboratory coat and glasses is recommended when assaying kit materials and samples.

Temperature:
Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

3. BACKGROUND

3.1 Acetylcholinesterase AChE® Technology

Acetylcholinesterase (AChE®), the enzymatic label for EIA, has the fastest turnover rate of any enzymatic label. This specific AChE is extracted from the electric organ of the electric eel, Electrophorus electricus, and it's capable of massive catalytic turnover during the generation of the electrochemical discharges. The use of AChE as enzymatic label for EIA has been patented by the French academic research Institute CEA [1, 2, 3].

AChE® assays are revealed with Substrate Solution (Ellman's reagent), which contains acetylthiocholine as a substrate. The final product of the enzymatic reaction (5-thio-2-nitrobenzoic acid), is bright yellow and can be read at 405-414 nm. AChE® offers several advantages compared to enzymes conventionally used in EIAs:

3.1.1 Kinetic superiority and high sensitivity

AChE® shows true fi rst-order kinetics with a turnover of 64,000 sec-1. That is nearly 3 times faster than Horse Radish Peroxidase (HRP) or alkaline phosphate. AChE® allows a greater sensitivity than other labeling enzymes.

3.1.2 Low background

non-enzymatic hydrolysis of acetylthiocholine in buffer is essentially absent. So, AChE® allows a very low background and an increased signal/noise ratio compared to other substrate of enzymes which is inherently unstable.

3.1.3 Wide dynamic range

AChE® is a stable enzyme and its activity remains constant for many hours as, unlike other enzymes, its substrate is not suicidal. This permits simultaneous assays of high diluted and very concentrated samples.

3.1.4 Versatility: AChE®

is a completely stable enzyme, unlike peroxidase which is suicidal. Thus, if a plate is accidentally dropped after dispatch of the AChE® substrate solution (Ellman's reagent) or if it needs to be revealed again, one only needs to wash the plate, add fresh Substrate Solution (Ellman's reagent) and proceed with a new development. Otherwise, the plate can be stored at +4°C with Wash Buffer in wals while waiting for technical advice from the Technical Support.

3.2 Ghrelin

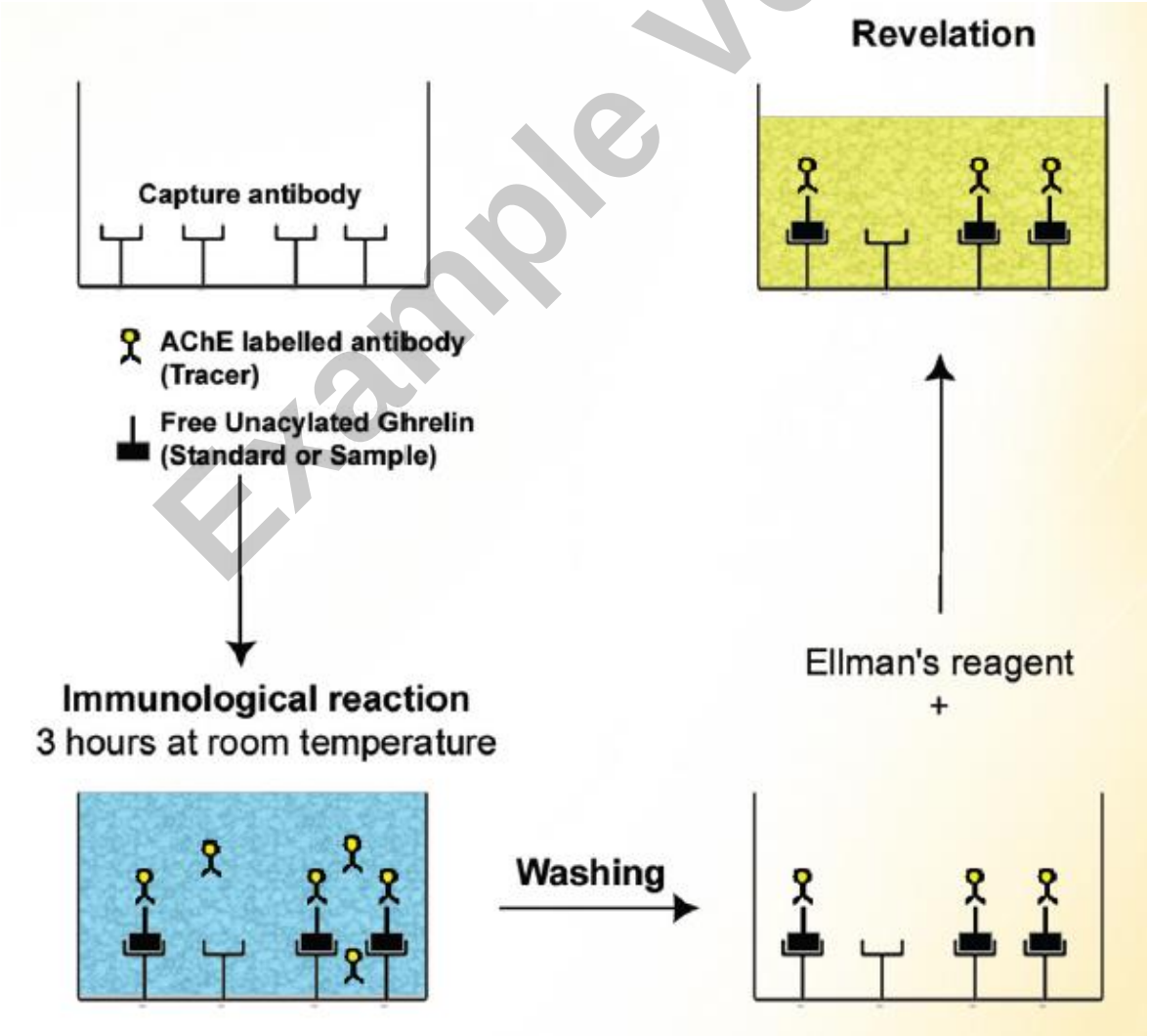
Ghrelin discovered in 1999, is fast becoming an endokrinology target of the millennium. Ghrelin, identifi ed in rat stomach as an endogenous ligand for the GH secretagogue receptor, is mainly produced in stomach, but has been demonstrated in many other organs [4, 5]. In addition to GH-releasing properties and its orexant action, Ghrelin could act as a hormone having effects on gastric motility (similarity with the peptide hormone motilin), acidic secretion, cardiovascular action, antiproliferative effects, pancreatic and glucose metabolism function, sleep [6, 7, 8].

Ghrelin gene raises to mRNA prepro-ghrelin of 117 amino acids. This precursor is processed into Ghrelin, 28 amino acids (human). Before being secreted, this peptide is octanoylated at Ser 3 by GOAT (Ghrelin Octanoyl Acyl Transferase). This step is Essentials for biological activity making GOAT a perfect target for drugs in feeding behaviour. Interestingly, the potential therapeutic importance of this hormone is not restricted to regulation of food intake [9] but also in cachexia (related to cancer treatment, anorexia nervosa or ischemia) [10] gastrin motility and may be involved in osteoporosis, somatopause, infertility and ovulation induction, neurological disorders (Alcoholism, Post Traumatic Stress disorders...) [11] and cardiovascular diseases.

4. PRINCIPLE OF THE ASSAY

This Enzyme Immunometric Assay (EIA) is based on a doubleantibody sandwich technique. The wells of the plate supplied are coated with a monoclonal antibody specifi c to the C-terminal part of Ghrelin.
This antibody will bind to any Ghrelin introduced into the wells (standard or sample). The acetylcholinesterase (AChE) - Fab' conjugate (Tracer) which recognises the N-terminal part of Unacylated Ghrelin is also added to the wells.
The two antibodies then form a sandwich by binding on different parts of the Unacylated Ghrelin. The sandwich is immobilised on the plate so reagents in excess may be washed away.
The concentration of Unacylated Ghrelin (mouse, rat) is determined by measuring the enzymatic activity of immobilized Tracer using Substrate Solution (Ellman's Reagent). AChE Tracer acts on Substrate Solution (Ellman's Reagent) to form a yellow compound that strongly absorbs at 414 nm.
The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of Unacylated Ghrelin (mouse, rat) present in the well during the immunological incubation.
This EIA so called Express EIA kit needs a short incubation time for immunological reaction (3 hours).

The principle of the assay is summarised below:



5. MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following material is required:

5.1 For sample preparation

– EDTA tubes for blood collection
– HCl 1N (optional)
– 35 sampling tubes with PHMB or reagents for PHMB inhibitor solution:
  Potassium Phosphate buffer 0.1 M pH 7.4
  NaOH 10N
  p-Hydroxymercuribenzoic acid (PHMB)
  UltraPure water (cat. number S0001)
  or Aprotinin (up to 0,6 TIU per ml blood)
  or PMSF

5.2 For the assay

– Precision micropipettes (20 to 1000 µl)
– Spectrophotometer plate reader (405 or 414 nm filter)
– Microplate washer (or wash-bottles)
– Orbital Microplate shaker
– Multichannel pipette and disposable tips 30-300 µl
– Ultra pure water
– Polypropylene tubes

Water used to prepare all EIA reagents and buffers must be Ultra Pure, deionized & free from organic contaminants traces.
Otherwise, organic contamination can significantly affect the enzymatic activity of the tracer AcetylCholinesterase. Do not use distilled water, HPLC-grade water or sterile water.

– Ultra pure water may be purchased from BioVendor

6. SAMPLE COLLECTION AND PREPARATION

This assay has been validated to measure Unacylated Ghrelin in buffer and in rat plasma samples.

6.1 General precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -20°C.

6.2 Blood Collection

- Blood samples are collected in tubes containing EDTA and a protease inhibitor to prevent the degradation of Acylated Ghrelin.
- Choice of protease inhibitor
  We suggest adding p-hydroxymercuribenzoic acid (PHMB) 1 mM in the final sample volume during the blood collection. We suggest preparing a 100 times concentrated solution of protease inhibitor and then adding 10 µl of this solution per ml of blood. For example, for PHMB 100mM concentrated solution, prepare a potassium phosphate buffer 0.1 M pH 7.4 in which 1.2% NaOH 10N volume/ volume is added. Then dissolve PHMB to get a 100 times concentrated solution (100 mM) in this buffer. Add 10 µl of this PHMB 100x solution per ml of blood. The PHMB 100x solution may be stored one month at -20°C.
  We suggest using aliquots for PHMB 100x solution in order to avoid freezing/thawing cycles.
  To avoid the preparation of PHMB protease inhibitor solution, BioVendor provides sampling tubes for 1 ml of blood containing PHMB. Other protease inhibitors could be used with the assay like Aprotinin (up to 0.6 TIU/ml blood) or PMSF(around 0.1 mg/ml blood according to literature) as indicated in the section “Protease inhibitor compatibility table” at the end of this booklet. For the use of these different products, please refer to the vendor’s instructions.
- Collection tubes are mixed by inversion 5 folds.
  Samples should be kept on ice between collection and centrifugation (15 minutes max).
- Blood samples are centrifuged at 3,500 rpm for 10 minutes at +4°C and then, supernatants are transferred in separate tubes. Samples should be quickly assayed or stored at -20°C for later use.
- The best way is to assay the samples within 3 weeks after the collection date. Moreover, we suggest using aliquots for plasma samples (we suggest 250 µl per aliquot) in order to avoid freezing/thawing cycles.
- **Acidification of freshly prepared plasma** (to be done before storage) **with HCl** is often performed and doesn’t affect the performance of the assay. When adding 100 µl of 1N HCl per ml of collected plasma and centrifuge them at 3.500 rpm for 5 min at +4°C, there is an increase on both Acylated Ghrelin value, and on Unacylated Ghrelin value. Poor neutralization (by dilution or NaOH) of acidification may however lead to inconsistency in sample reproducibility.
- Plasma samples prepared as above-mentioned can be assayed for Acylated Ghrelin with Acylated Ghrelin EIA kit or for Unacylated Ghrelin with Unacylated Ghrelin EIA kit.

6.3 Sample preparation

Plasma samples may be assayed directly without any extraction procedure after being diluted at **least to 1:10 in Dilution Buffer** in order to avoid matrix effect.

7. REAGENT PREPARATION

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 36 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of Standard, one of Quality Control and one of Substrate Solution.

All reagents need to be brought to room temperature, around +20°C, prior to the assay.

7.1 Dilution Buffer

Reconstitute the vial Dilution buffer with 50 ml of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at 4°C: 1 month

7.2 Unacylated Ghrelin (mouse,rat) Standard

Reconstitute the Standard vial with 1 ml of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

The concentration of the first standard S1 is 250 pg/ml.

Prepare seven propylene tubes for the other standards and add 500 µl of Dilution Buffer into each tube. Then prepare the standards by serial dilutions as follows:

Standard	Volume of Standard	Volume of Dilution Buffer	Standard concentration pg/ml
S1	-	-	250
S2	500 µl of S1	500 µl	125
S3	500 µl of S2	500 µl	62.5
S4	500 µl of S3	500 µl	31.3
S5	500 µl of S4	500 µl	15.6
S6	500 µl of S5	500 µl	7.8
S7	500 µl of S6	500 µl	3.9
S8	500 µl of S7	500 µl	2.0

Stability at +4°C: 1 week

7.3 Unacylated Ghrelin (mouse, rat) Quality Control

The Quality Control provided in this kit has been prepared by spiking Unacylated Ghrelin (rat) peptide in Dilution Buffer.
Reconstitute the Quality Control vial with 1 ml of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at +4°C: 1 week

7.4 Unacylated Ghrelin Conjugate Solution

Reconstitute the vial Conjugate Solution with 10 ml of Dilution Buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at +4°C: 1 week

7.5 Wash Buffer

Dilute 2 ml of concentrated Wash Buffer with 800 ml of UltraPure water. Add 400 µl of Tween20. Use a magnetic stirring bar to mix the content.
Stability at +4°C: 1 week

7.6 Substrate Solution (Ellman's Reagent)

5 minutes before use (development of the plate), reconstitute one vial of Substrate Solution (Ellman's Reagent_49+1) with 49 ml of UltraPure water and 1 ml of concentrated Wash Buffer. The tube content should be thoroughly mixed.
Stability at 4°C and in the dark: 24 hours

8. ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

8.1 Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section. Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.
Stability at +4°C: 1 month.
Rinse each well 5 times with the Wash Buffer 300 µl/well.
Just before distributing reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

8.2 Distribution of reagents and samples

A plate set-up is suggested hereafter.
The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	S7	S3	*	*	*	*	*	*	*	*	*
B	B	S7	S3	*	*	*	*	*	*	*	*	*
C	B	S6	S2	*	*	*	*	*	*	*	*	*
D	NSB	S6	S2	*	*	*	*	*	*	*	*	*
E	NSB	S5	S1	*	*	*	*	*	*	*	*	*
F	NSB	S5	S1	*	*	*	*	*	*	*	*	*
G	S8	S4	QC	*	*	*	*	*	*	*	*	*
H	S8	S4	QC	*	*	*	*	*	*	*	*	*

B : Blank

S1-S8: Standards 1-8

* : Samples

NSB : Non Specific Binding

QC: Quality Controls

8.3 Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.
Use different tips to pipette the buffer, standard, sample, conjugate, antiserum and other reagents.
Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expeling with the pipette tip.

8.3.1 Dilution Buffer

Dispense 100 µl to Non Specific Binding NSB wells.

8.3.2 Unacylated Ghrelin (mouse, rat) Standards

Dispense 100 µl of each of the eight standards S1 to S8 in duplicate to appropriate wells.
Start with the lowest concentration standard S8 and equilibrate the tip in the next higher standard before pipetting.

8.3.3 Quality Control and samples

Dispense 100 µl in duplicate to appropriate wells. Highly concentrated samples may be diluted in Dilution Buffer.

8.3.4 Unacylated Ghrelin Conjugate Solution

Dispense 100 µl to each well, **except** Blank (Bk) wells.

8.4 Incubating the plate

Cover the plate with the cover sheet and incubate for 3 hours at room temperature.
A longer immunological reaction (20 hours at +4°C) is also possible, increasing the sensitivity of the assay to 0.6 pg/ml.

8.5 Developing and reading the plate

- Reconstitute Substrate Solution (Ellman's reagent) as mentioned in the Reagent preparation section.
- Empty the plate by turning over. Rinse each well five times with 300 µl Wash Buffer. The 5th time, slightly shake the plate for 5 minutes on an orbital shaker. Then rewash five times with 300 µl Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.
- Add 200 µl of Substrate Solution (Ellman's reagent) to each 96 well. Cover the plate with aluminium sheet and incubate in the dark at room temperature. Optimal development is obtained using an orbital shaker.
- Wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.
- Read the plate at a wavelength between 405 and 414 nm (yellow colour).

After addition of Substrate Solution (Ellman's reagent), the absorbance has to be checked periodically (every 30 minutes) until the maximum absorbance has reached a minimum of 0.5 A.U. blank subtracted.

Express Enzyme Immunoassay Protocole (volumes are in µL)				
	Blank	**NSB**	**Standard**	**Sample or QC**
EIA Buffer	-	100	-	-
Standard	-	-	100	-
Sample or QC	-	-	-	100
Tracer	-	100	100	100
Cover plate, incubate 3 hours at RT				
Wash plate 5 times, shake 5 min, wash 5 times & discard from the wells				
Ellman´s reagent	200			
Incubate with an orbital shaker in the dark at RT				
Read the plate between 405 and 414 nm				

9. DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of Substrate Solution (Ellman's reagent) alone) from the absorbance readings of the rest of the plate. If not, do it now.

- Calculate the average absorbance for each NSB, standard and sample.
- For each standard, plot the absorbance on y axis versus the concentration on x axis. Draw a best-fit line through the points.
- To determine the concentration of your samples, find the absorbance value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of your unknown sample. Do not forget to integrate the dilution factor of your own samples (due notably to the minimal dilution for the assay 1:10 and the addition of HCl 1N).
- Samples with a concentration greater than 250 pg/ml should be re-assayed after dilution in Dilution Buffer.
- Most plate readers are supplied with curve-fitting software capable of graphing these data (logit/log or 4-parameter logistic fi t 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

Two vials of Quality Control are provided with this kit. Your standard curve is validated only if the calculated concentration of the Quality Control obtained with the assay is +/- 25% of the expected concentration (written on the Quality Control Sheet)

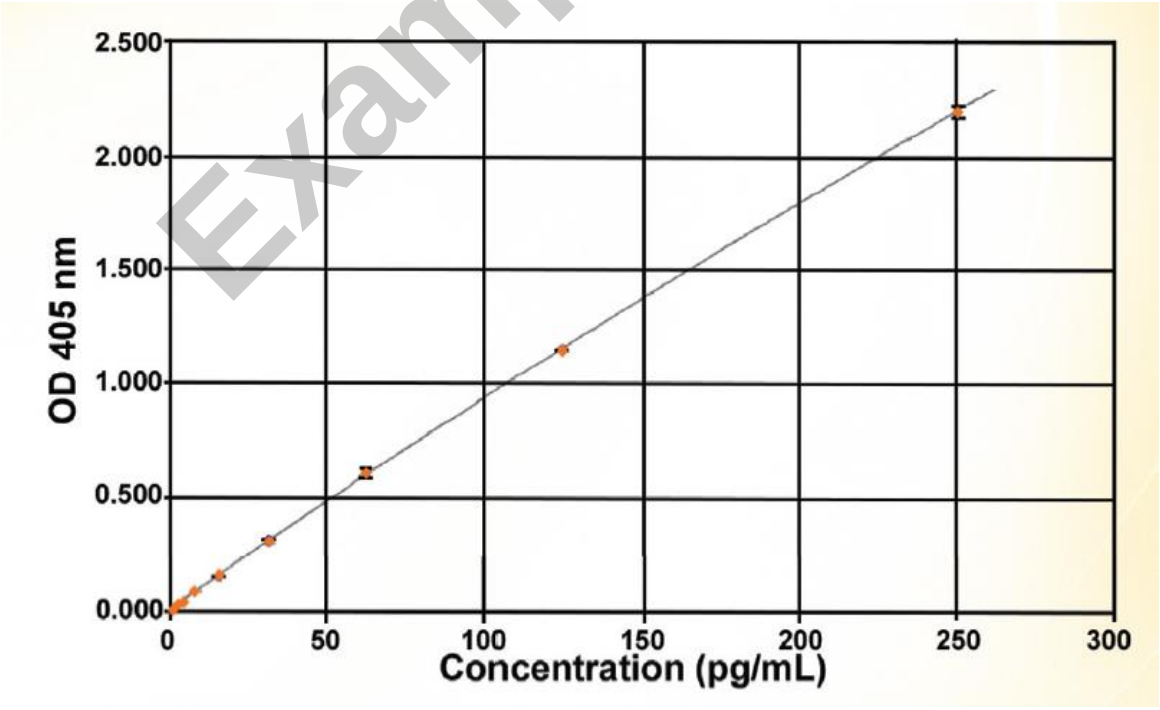
10. ACCEPTABLE RANGE

- Non Specific Binding < 60 mA.U.
- Limit of detection in the sample before dilution <8 pg/ml
- QC sample: ±25% of the expected concentration (see on the Quality Control Sheet)

11. TYPICAL RESULTS

The following data are for demonstration purpose only. Your data may be different and still correct. These data were obtained using all reagents as supplied in this kit under the following conditions: 60 minutes developing, fading at 414 nm. A 4-parameter logistic fitting was used to determine the concentrations.

	Unacylated Ghrelin (mouse, rat) pg/ml	Absorbance (mAU)
Standard S1	250	2196
Standard S2	125	1155
Standard S3	62.5	611
Standard S4	31.3	315
Standard S5	15.6	157
Standard S6	7.8	93
Standard S7	3.9	56
Standard S8	2.0	37



Typical Unacylated Ghrelin (mouse, rat) standard curve

12. ASSAY VALIDATION AND CHARACTERISTICS

Immunometric assay of Unacylated Ghrelin (mouse, rat) has been validated for its use in buffer and in plasma (without extraction but diluted at least 1:10). A sigmoidal 4-parameter logistic fitting was used to determine the concentrations.
For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography [12].

12.1 Limit of detection

Calculated as the concentration of Unacylated Ghrelin corresponding to the NSB average (n = 8) plus three standard deviations is 0.8 pg/ml. Due to the minimal plasma dilution (1:10), the limit of detection in the samples is less than 8 pg/ml.

12.2 Intra-assay & inter-assay variations and recovery:

QC levels	Theoretical concentrations in diluted QC (pg/ml)	Mean of observed concentrations (pg/ml)	Intra-assay (CV%)	Inter-assay (CV%)	Recovery (%)	Confidence intervalle (α= 0.05)
Incubation 3 hours at room temperature						
QC1	2	2.3	19.5	19.8	117.0	117.0 ± 9.3
QC2	25	22.7	5.2	5.5	90.6	90.6 ± 2.1
QC3	200	184.0	2.9	3.3	91.8	91.8 ± 1.6
Incubation 20 hours at +4°C						
QC1	2	2.4	15.9	15.9	120.0	120.0 ± 7.3
QC2	25	22.8	4.8	5.5	91.2	91.2 ± 2.6
QC3	200	187	4.0	4.3	93.3	93.3 ± 1.9

The intra-assay and inter-assay variations were studied on pool of rat plasma (free of Ghrelin). Each level of QC was prepared ten times concentrated from this pool of rat plasma and then diluted to 1:10 in Dilution Buffer before assay. 60 aliquots were prepared for each of 3 level of QC. Replicate samples (n=6) at each of the three validation levels were analysed along with the calibration curve for a total of 5 independent runs.

12.3 Matrix variability

Matrix	Theoretical concentration (pg/ml)	Unacylated Ghrelin measured (pg/ml)	Recovery (%)	Mean of recovery (%)
1	25	25.9	104.0	103.0
2		27.3	109.0	
3		25.8	103.0	
4		24.7	98.8	
5		24.7	98.8	

Five individual lots of rat plasma samples were tested.
Validation samples (n=3) were prepared five times concentrated in each matrix (free from Ghrelin) and then diluted to 1:10 in order to obtain a final concentration of 25 pg/ml. QC were analysed against a calibration curve derived from a pool of rat plasmas.

12.4 Dilution tests

Samples	Dilution Factor	Unacylated Ghrelin measured (pg/ml)	Corrected concentrations (pg/ml)	Recovery (%)	Mean of recovery (%)
1	1:10	79.2	792	-	104
	1:20	41.0	820	104	
	1:50	17.0	850	107	
	1:100	7.8	784	99	
	1:200	4.1	828	105	
2	1:10	86.8	241	-	105
	1:20	44.1	244	101	
	1:50	18.1	252	105	
	1:100	8.6	255	106	
	1:200	4.3	259	107	
3	1:10	83.6	836	-	96.7
	1:20	42.7	854	102	
	1:50	16.7	835	100	
	1:100	7.7	774	93	
	1:200	3.9	772	92	

Three rat plasma samples were diluted to 1:10. Afterwards, four independent dilutions (n=3) were performed and analysed against a calibration curve.

12.5 Stability tests

Samples	Reference value (pg/ml)	1 cycle (pg/ml)	2 cycles (pg/ml)	3 cycles (pg/ml)	Mean of recovery (%)
1	771	803	820	727	101.6
2	617	602	632	563	97.1
3	668	616	642	597	92.6
4	749	689	700	660	91.2
5	838	784	722	715	88.3

Five rat plasma samples (n=3) were analysed just after collection and dilution to 1:10 before the assay (reference value) and after 1, 2 and 3 freeze/thaw cycles.

12.6 Cross-reactivity

Unacylated Ghrelin (human)	100%
Acylated Ghrelin (mouse, rat)	<0.001 %
Acylated Ghrelin (human)	<0.001 %
Ghrelin (1-14) (human)	<0.001 %
Ghrelin (1-11) (rat)	<0.001 %
Ghrelin (17-28) (human, rat)	<0.001 %
GHRF (rat)	<0.001 %
Insulin (rat)	<0.001 %
Motiline	<0.001 %
Leptin (rat)	<0.001 %
Somatostatine	<0.001 %
CRF (human, rat)	<0.001 %
Glucagon (human, rat)	<0.001 %

12.7 Protease Inhibitor compatibility table

	AEBSF	PMSF	Pefabloc	P800	Aprotinin	PHMB
RA394063400R	NO	YES	NO	NO	YES	YES

Plasma samples were collected on different protease inhibitors according to vendors instruction and measured with the appropriate kit. Recovery is different from one inhibitor to the other and it belongs to the end user to defi ne according to its Leeds which inhibitor to be used. Acidification has also been tested with most inhibitors and may also change recovery but will not affect the assay performances providing that dilution with Dilution Buffer or neutralisation is performed.

13. ASSAY TROUBLESHOOTING

13.1 Absorbance values too low:

- organic contamination of water
- one reagent has not been dispensed
- incorrect preparation/dilution
- assay performed before reagents reached room temperature
- reading time not long enough

13.2 High signal and background in all wells:

- inefficient washing
- overdeveloping (incubation time should be reduced)
- high ambient temperature

13.3 High dispersion of duplicates:

- poor pipetting technique
- irregular plate washing

13.4 If a plate is accidentally dropped after dispatch of the AChE® substrate solution (Ellman's reagent) or if it needs to be revealed again:

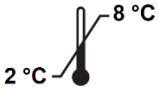
- one only needs to wash the plate, add fresh Substrate Solution and proceed with a new development
- otherwise, the plate can be stored at +4°C with wash buffer in wells while waiting for technical advice from the Technical Support

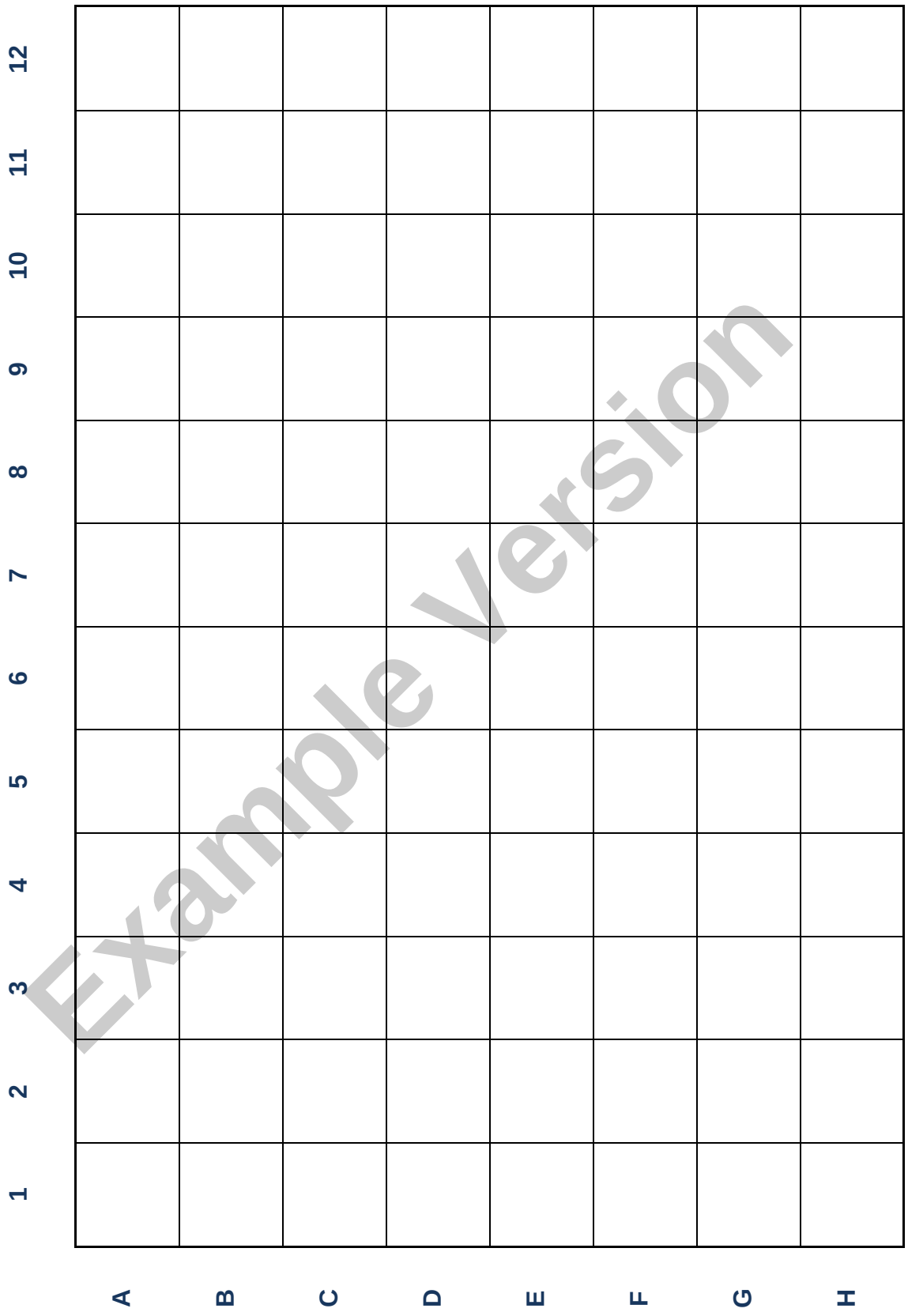
14. REFERENCES

1. Grassi J, Pradelles P
   Compounds labelled by the acetylcholinesterase of Electrophorus Electricus. Its preparation process and its use as a tracer or marquer in enzymo-immunological determinations. United States patent, N° 1,047,330. September 10, 1991
2. Grassi J, Pradelles P
   The use of Acetylcholinesterase as a Universal marker in Enzyme-Immunoassays
   Proceedings of the Third International Meeting on Cholinesterases, American Chemical Society (1991)
3. Pradelles P, Grassi J, Maclouf J
   Enzyme Immunoassays of Eicosanoids Using Acetylcholinesterase
   Methods in Enzymology (1990), vol. 187, 24-34
4. Kojima M, Kangawa KGhrelin: structure and fiction
   Physiol. Rev (2005), 85:495-522,
5. Bluet-Pajot MT, Tolle V, Zizzari P, Tomasetto C, Grouselle D, Epelbaum J. Ghrelin: A striking example of neuroendocrine peptide pleiotropy
   Med Sci (Paris), August 1, 2005, 21 (8-9): 715-21
6. de Faria Barros A et al
   Is there association between acyl-ghrelin and infl ammation in hemodialysis patients?
   J Bras Nefrol. (2013) 35(2):120-126.
7. Grousselle D et al Variations des peptides dérivés de la préproghréline au cours du repas dans l'anorexie mentale restrictive
   Poster GIR-AFDAS-TCA2014
8. Delhanty P et al
   Des-acyl ghrelin analogs prevent high-fat-diet-induced dysregulation of glucose homeostasis
   FASEB J. (2013) 27(4):1690-1700.
9. Costantini, V et al
   GSK1614343, a Novel Ghrelin Receptor Antagonist, Produces an Unexpected Increase of Food Intake and Body Weight in Rodents and Dogs
   Neuroendocrinology (2011); 94:158–168
10. Porporato E, Filigheddu N et al
    Acylated and unAcylated Ghrelin impair skeletal muscle atrophy in mice.
    J. Clinical Invest (2013) 123(2): 611-622
11. Sentissi O, Epelbaum J, Olié JP, Poirier MF
    Leptin and Ghrelin Levels in Patients With Schizophrenia During Different Antipsychotics Treatment: A Review
    Schizophrenia Bulletin (2008) 34(6), 1189–1199
12. Valentin MA, Ma S, Zhao A, Legay F, Avrameas A
    Validation of immunoassay for protein biomarkers: Bioanalytical study plan implementation to support pre-clinical and clinical studies.
    J Pharm Biomed Anal. (2011) 55(5 : 869-877
13. European Medicines Agency

    Guideline on bioanalytical method validation, 21 July 2011

15. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



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