

# Ghrelin – RIA

Radioimmunoassay for quantitative Determination of  
**human Ghrelin**  
English

For research and professional use only!



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## Symbols / Symbole

according to DIN EN 980 and EDMA recommendations Standard News 6 2001  
EN/ DE/ FR/ IT/ ES/ PT/ NL/ DK/ SE/ PL/ HU/ SK/ CZ/ BG/ EE/ GR/ RO/ SL/ FI

## Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ Symbolen/ Symboler/ Symboler/ Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit

according to DIN EN 980 and EDMA recommendations Standard News 6 2001



Expiry date/ Verfallsdatum/ Date de péremption/ Data di scadenza/ Fecha de caducidad/ Data de validade/ Uiterste gebruiksdatum/ Udløbsdato/ Bäst före-datum/ Termin ważności/ Lejárati idő/ Čas expirácie/ Doba expirace/ Срок на годност/ Aegumiskuupäev/ Ημερομηνία λήξης/ Data de expirare/ Rok uporabe/ Viimeinen käyttöpäivä



Consider instructions for use/ Bitte Gebrauchsanweisung beachten/ Consultez la notice d'utilisation/ Consultare le istruzioni per l'uso/ Consulte las instrucciones de uso/ Respeitar as instruções de utilização./ A.u.b de gebruiksaanwijzing volgen/ Se brugsanvisningen/ Läs anvisningarna före användning/ Proszę przeczytać instrukcję obsługi/ Vegye figyelembe a használati utasításban foglaltakat/ Postupujte podľa pokynov na použitie/ Dodržujte návod k použití/ Моля, спазвайте инструкцията за употреба/ Palun järgige kasutusjuhendit./ Λάβετε υπόψη σας τις οδηγίες χρήσης/ Vá rugám sã respectați instrucțiunile de utilizare/ Upošteвайте navodila za uporabo/ Lue käyttöohje huolellisesti!



Lot-Batch Number/ Charge-Chargennummer/ Lot-Code du lot/ Lotto-Numero di lotto/ Lote-Código de lote/ Lote-Código do Lote/Lot-Partijnummer/ Lot-Batchkode/ Partisatskod/ Numer serii/ Tétel-sarzs szám/ Číslo šarže/ Číslo šarže/ Партиден номер/Partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ erä



Manufactured by/ Hergestellt von/ Fabriqué par/ Prodotto da/Fabricado por/ Fabricado por/ Vervaardigd doo/Fabrikation af /Tillverkad av/ Wyprodukowane przez/ Gyártotta / Vyrobené/ Vyrobeno v/ Производител/ Τοοτjα/ Κατασκευάζεται από/ Produs de/ Proizvajalec/ Valmistaja



Catalogue Number/ Bestellnummer/ Numéro de référence/Numero di riferimento/ Número de referencia/ Número de Referência/ Referentienummer/ Referencenummer /Beställningsnummer/ Numer katalogowy/ Rendelési szám/Katalógové číslo/ Objednací číslo/Каталожен номер/Tellimisnumber/ Αρ. παραγγελίας/Număr comandă/ Številka naročila/ viite tai tilausnumero



Store at between/ Lagerung bei zwischen/ Conserver à entre/ Conservare a tra/ Conservar a temp. Entre/ Armazenaer entre/ Bewaar bij tussen/ Opbevaars mellem/ Förvaras vid/ Przechowywać w/ Tárolási tartomány/ Skladujte v rozsahu / Skladujte v rozmezi/ Температурно ограничение/ Säilittä temperatuuridel/ Φύλαξη σε θερμοκρασία/ Depozitare între/ Skladiščenje med/ Säilytys x-y Celsiusasteen lämpötilassa



Contains sufficient for x tests/ Inhalt ausreichend für x Tests/ Contient suffisant pour x tests/ Contenuto sufficiente per x test/ Contenido suficiente para x pruebas/ Conteúdo suficiente para x testes/ Bevat voldoende voor x bepalingen/ Indeholder tilstrækkeligt til x prøver/ Innehållet räcker till x analyser/ Zawartość na x testów/ Tartalma x teszt elvégzésére elegendő/ Obsahuje materiál pre x testov / Obsah dostacuje pro x testů/ Съдържание достатъчно за x тестове/ Sisust jätkub x katse jaoks/ Το περιεχόμενο επαρκεί για x δοκιμές/ Conținut suficient pentru x teste/ Vsebina zadostuje za x preizkusov/ Sisältö riittää x testille



Keep away from sunlight/ Nicht dem Sonnenlicht aussetzen/ Conserver à l'abri de la lumière/ Conservare al riparo della luce solare/ No exponer a la luz solar/ Proteger da luz solar/ Niet aan zonlicht blootstellen/ Må ikke udsættes for sollys/ Utsätt inte för solljus/ Nie wystawiać na słońce/ Napfénytől távol tartandó/ Nevystavovat slnečnému svetlu/ Nevystavovat slnečnému svétlu/ Да се предпазва от слънчева светлина/ Kaitsta otsese päikesekiirguse eest/ Κρατήστε το μακριά από την ηλιακή ακτινοβολία/ Τηνετj departe de lumina soarelui/ Ne izpostavljajte sončni svetlobi/ suojaa auringonvalolta



Radioactive /radioaktiv/ Radioactif/ Radioattivo/ radioactivo/ radioactief/ radioaktiv/radioaktiv/ radioaktywny /radioaktív/ rádioaktívne/ radioaktivni/ радиоактивно/ radioaktiivne/ Ραδιενεργό/radioactive/ radioaktivno/ radioaktiivinen



Incubation time/ Inkubationszeit/ Temps d'incubation/ Tempo d'incubazione/ Tiempo de incubación/ Tempo de incubação/ incubatietijd/Inkubationstid/ inkubationstid/ Czas inkubacji/ Inkubációs idő/ Inkubační lehota/ Inkubační doba/ Инкубационен период/ Inkubatsiooniaeg/ Χρόνος επώασης/ Timp de incubare/ Inkubacijska doba/ inkubaatioaika



Incubate at / Inkubation bei/ Incuber à/ Incubare a/incubar a/Incubar a/ incubatietemperatuur/ Inkubation ved/ inkubation vid/ Inkubacja przy/ Inkubáció hőmérséklete/Inkubácia pri/ Inkubace při/Инкубира се при/ Inkubatsioon temperatuuril/ Επώαση στους/ Incubare la/ Inkubacija pri/ inkubaatiolämpötila



Mix tubes with a Vortex mixer/ Mix Rührchen mit Vortex Mixer/ Mélanger à l'aide d'un vortex/ Miscelare la provetta con agitatore Vortex/ Tubos de mezcla con mezclador de vortex/ Misturar os tubos com um agitador Vortex/ buisjes mengen met een Vortex/ Blanderør med Vortex-mixer/ Blanda rören med en vortexblandare/ Miksowanie rurek w mikserze Vortex/ Csövecskék keverése örvénykeverővel/ Premiešať pomocou prístroja Vortex/ Promíchat pomocí přístroje Vortex/ Разбъркване на епруветките с миксер Vortex/ Segada torukesi Vortexi mikseriga/ Αναμίξτε τους σωληνίσκους με αναδευτήρα Vortex/ Amestecați eprubetele cu ajutorul unui agitator vortex/ Mešanje cevčic z mešalnikom Vortex/ Sekoita putket Vortex sekoittajalla



Centrifuge/ Zentrifugieren/ Centrifuger/ Centrifugare/ Centrifugar /centrifugeren/ Centrifugering/ centrifugera/ Odwirowywanie/ centrifugálás/ odstredit/ odstfedit/ Центрофугиране/ Tsentrifuugida / Φυγοκέντρηση/ centrifugare/ centrifugirati/ sentrifugoi



All/ alle/ Tout/ Tutto/ Todo/ tudo/ alle/ alle/ alla/ wszystkie/ minden/ všetko/ všechno/всички/kõik/ όλα/ toate/ysi/kaikki



Not/ Nicht/ Non/ Non/ No/ Não/ niet/ Ikke/ inte/ nie/ Nem/ не/ mitte / όχι / Nu /Ne/ ei

maximal Binding/ maximale Bindung/ Liaison maximale/ legame max./ Enlace máximo/ Ligação máxima/ maximale binding/ maksimal binding / maximal bindning/ maksymalne wiązanie/maximális kötés/ Maximálne väzby/ Maximální vazby/ максимално свързване/ maksimaalne sidumine/ Μέγιστη δέσμευση/ legäturä maximä/ Maksimaalna vez/ Maksimaalinen sidos

<b>TC</b>		Total Counts / Total Counts/ Coups totaux/ Conte totali/ Conteo total/ Total de Contagens/ totaaltellingen/ Samlede tællinger/ totalt antal/ Licznik całkowity/ Teljes szám/ Celkový počet/ Celkový počet/ Общo количество/ Σύνολο αποκλιών / Număr total/ Skupno število /Kokonaissykäykset
<b>Tube</b>		Streptavidin-coated Tubes / Streptavidin-beschichtete Röhrchen/ tubes revêtus de streptavidine/ Provette rivestite di streptavidina/ Tubos recubiertos con estreptavidina/ Tubos revestidos com Streptavidina/ buisjes met streptavidin gecoat/ Streptavidin-coatede rør/ streptavidinbelagda rör/ Rurki z powłoką streptawidynowym/ Streptavidin-bevonatú csövecskek/ Rúrky s vrstvou streptavidínu Trubičky s vrstvou streptavidínu/ Наговарени със стрептавидин епруветки/ Streptavidiniiga kaetud torukesed/ Σωληνίσκοι επικαλυμμένοι με στρεπταβιδίνη/ Erprobete acoperite Streptavidin/ Cevčice, prekrite s streptavidinom/ Streptavidiniilla pinnoitetut putket
	T	
<b>Rec in</b>		Reconstitute in/ Rekonstituieren in/ Reconstituer dans/ Ricostituire nel/ Reconstituir en/ Reconstituir em/ reconstituieren in/ Rekonstituér i/ rekonstituera/ Rekonstytuować w/ Helyreállítás/ Znovu pripraviť za/ Znovu připravit za/ Разтваряне в/ Moodustada uuesti / Ανασυστήστε σε/ Reconstituire în/ Predelava v/ rekonstituoi
<b>SPE</b>		Sample/ Probe /Echantillon/ campione/ Muestra/ Amostra/ monster/ Prøve/ prov/ Próbka/ Minta/ Vzorka/ Vzorek/ Προβα/ Proov/ Δείγμα/ Probá/ Vzorec/ Näyte
<b>BUF</b>	X	AB, PR Buffer/ Puffer/ Tampon/ tampone/ Tampón/ Tampão/ buffer/ Buffer/ buffert/ Bufor/ Puffer/ Puffer/ Puffer/ Буфер/ Puhver/ Ρυθμιστικό διάλυμα/ tampon/ Puffer/ Puskuri
<b>Tracer</b>		C Tracer / Tracer/ Traceur/ Tracciatore/ Trazador/ Marcador/ tracer/ Tracer/ spårämne/ Indykator/ Nyomjelző/ Indikátor/ Indikátor/ Трейсер/ Kandur/ Ιχνηθέτης/ Indicator/ Sledilna snov/ indikaattori
<b>2.Ab</b>		2.Ab Capture Antibody/ Fang-Antikörper/ Anticorps de capture/ Anticorpo di cattura/ Anticuerpos de captura/ Anticorpo de captura/ vanger-antilichaam/ Fæstet antistof/ fångande antikropp/ Przechwytywanie antycial/ Elfogó antitest/ Zachytenie protilátky/ Zachycení protilátky/ Захващащо анти тяло/ Haarde-antikehad/ Ακίνητοποιημένο αντίσωμα/ Captură anticorpi/ Lovilna protitelesa / sekundäärinen vasta-aine
<b>1.Ab</b>		1.Ab Specific Antibody/ spezifischer Antikörper/ Anticorps spécifique/ Anticorpo specific/ Anticuerpos específicos/ Anticorpo específico/ speciefike antilichaam/ Specifisk antistof/ specifik antikropp/ Określone antyciała/ specifikus antitest/ Špecifická protilátka/ Špecifická protilátka/ Специфично анти тяло/ eriomased antikehad/ Ειδικό αντίσωμα/ anticorpi specifici/ Specifična protitelesa/ Spesifinen vasta-aine
<b>DILU</b>	X	Dilute in Buffer X/ Verdünnen in Puffer X/ Diluer dans le tampon X/ Diluire nel tampone X/ Diluir en tampón X/ Diluir no Tampão X/ verdunnen in buffer X/ Fortyndes i buffer X/ späđ i buffert X/ Rozcieńczenie w buforze X/ Hígítás X pufferben/ Riedit' v puffri X/ Ředit v puffru X/ Разреждане в буфер X/ Lahjendada puhvris X/ Αραιώστε σε ρυθμιστικό διάλυμα X/ Diluați în tamponul X/ Razredčiti v puffru X/ laimennetaan x puskuriin
<b>CAL</b>	X	Std. 1-6 Standard X /Standard X/ Etalon X/ Standard X/ Estándar X/ Standard X/ standaard X/ Standard X/ standard X/ Standard X/ Standard X/ Štandard X/ Standard X/ Стандарт X/ Standard X/ Πρότυπο X/ Standard X/ Standardni X/ Kalibraattori X
<b>Control</b>		CS Control Serum / Kontrollserum/ Contôle sérique/ Siero di controllo/ Suero de control/ Soro de Controlo/ controleserum/ Kontrolserum/ Kontrollserum/ Serum kontrolne/ Ellenőrző szérum/ Kontrolné sérum/ Kontrolní sérum/ Контролен серум/ Kontrollseerum/ Ορός ελέγχου/Ser de control/ Kontrolni serum/ Kontrolli seerumi
<b>MEASURE</b>		Count radioactivity of all Tubes/ Radioaktivität aller Röhrchen messen / Compter l'activité de tous les tubes/ Misurare la radioattività di tutte le provette/ Medir la radioactividad de todos los tubos/ Medir a radioactividade de todos os tubos/ radioactiviteit van alle buisjes meten/ Mål radioaktivitet for alle rør/ Mät radioaktiviteten i alla rör/ Zmierzyć radioaktywność wszystkich rurek/ Minden csövecske radioaktivitásának mérése/ Merať rádioaktivitu všetkých rúrok/ Měřit radioaktivitu všech trubiček/ Измерване на радиоактивността на всички епруветки/ Kõigi torukeste radioaktiivsuse mõõtmine / Μετρήστε τη ραδιενέργεια όλων των σωληνίσκων / Măsurati radioactivitatea tuturor eprubetelor / Meritev radioaktivnosti vseh cevčic / Mittaa kaikkien putkien radioaktiivisuus
<b>Literatur</b>		Literature/ Literatur/ Bibliographie/ Letterario/ Bibliografia/ Literatura documentação/ literatuur/ Litteratur/ litteratur/ Literatura/ Irodalom/ Literatura/ Literatura/ Литература// Kirjandus/ Βιβλιογραφία/ Bibliografie/ literatura/ Lähdeluettelo
<b>International Test description</b>		International test description/ internationale Testanleitung/ description internationale de test/ Istruzioni per il test internazionali/ Descripción de ensayo internacional/ Descrição internacional do teste/ internationale testbeskriving/ internationell testbeskrivning/ Opis testu międzynarodowego/ nemzetközi teszt-útmutató/ Medzinárodný návod k testu/ Mezinárodní návod k testu/ rahvusvaheline katse kirjeldus/ Διεθνείς οδηγίες για εργαστηριακές δοκιμές/ instrucțiuni internaționale pentru testare/ mednarodna navodila za preizkus/ Kansainvälinen käyttöohje

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<b>Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ Symbolen/ Symboler/ Symboler/ Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit</b>	<b>2</b>
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## TECHNICAL FEATURES+APPLICATIONS

- ◆ For Research and Professional Use Only!
- ◆ analytical sensitivity 0.04 ng/ml
- ◆ Intra- and Inter Assay Variance < 10%
- ◆ Recovery of recombinant Ghrelin 97%
- ◆ Control Serum included
- ◆ For measurement in human serum

## INTENDED USE

This radioimmunoassay kit is intended for research and professional use and quantifies human Ghrelin in serum and EDTA-Plasma.

## INTRODUCTION

Ghrelin is a 3.5 kDa protein of 28 amino acids and is serine octanylated. Bioactivity of this small peptide hormone depends on octanylation (1). It is mainly synthesized by stomach but also in duodenal and heart cells (2) and therefore indicates the relevance of the stomach as endocrine organ (3).

Ghrelin is able to cross the blood-brain barrier and is a natural ligand of growth hormone secretagogue receptor in pituitary and hypothalamus (4). Ghrelin exert influence on several neurological processes, for instance memory retention can be modulated by ghrelin (5) and Ghrelin secretion is influenced by sleep (6, 7). Not only growth hormone but several other hormones are influenced by Ghrelin e.g. ACTH, cortisol, prolactin (8, 9). It is also present in pancreatic islets and regulates insulin secretion (10-12). In women with polycystic ovary syndrome Ghrelin levels are decreased and highly correlated to insulin sensitivity (24), so there are several regulatory circles influenced by Ghrelin. Additionally to endocrine action Ghrelin exerts influence on immunological processes. In Human umbilical vein endothelial cells Ghrelin inhibits basal and TNF-alpha-induced cytokine release and mononuclear cell binding and in vivo endotoxin-induced proinflammatory cytokine production in rats was also inhibited by intravenous administrated Ghrelin (13).

Sites of Ghrelin synthesis as well as receptor location indicate a role for the hormone and the gut in appetite regulation (1) (23). So many Ghrelin receptors are present in the hypothalamic arcuate nucleus, a brain area important in food intake control. Several investigations demonstrate a circadian rhythm of Ghrelin secretion (14), controlled by ingestion. Shortly before food intake Ghrelin plasma concentration increases and decreases after finishing. In eating disorders Ghrelin levels reflect illness, so obesity suppresses Ghrelin concentration in blood (14) and in anorexia nervosa an increase of Ghrelin serum concentration can be detected (15-17). Ghrelin might act as counterpart to Leptin in the regulation of food intake and fat utilization (16). It also influences the adipogenesis negatively (18). A significant decrease of Ghrelin concentration is detected in elderly people (19). This could explain the anorexia of elderly people and offers a new target in anti-aging research.

So Ghrelin seems to be involved in the regulation of many physiological processes and its influence on many of these processes has not been investigated in detail. With this test system we provide an easy but reliable tool for the quantification of human Ghrelin in serum.

## PRINCIPLE

For the Radioimmunoassay for the determination of human Ghrelin a polyclonal rabbit-antibody of high specificity is used. Ghrelin is measured quantitatively.

Standards are prepared from recombinant Ghrelin, <sup>125</sup>I-Tracer from a C-terminated peptide (AS 15 – 28) with presynthesised Tyrosine.

## Calibration of the Assay

The assay was calibrated against the internal test of Medical School Hannover, Prof. Brabant.

## PERFORMANCE CHARACTERISTICS

### Sensitivity

The analytical **sensitivity** of the assay yields **40 pg/ml (about 10 pmol/L)** measured as 2x SD of zero standards.

### Specificity

This assay is specific for human Ghrelin. The antibody shows in addition cross-reactivity with: rabbit, cat, guinea pig, hamster, goat, sheep, rat, horse, donkey, pig, dog, rabbit, mouse and bovine. No cross-reactivity was found with other proteins such as insulin or GH.

**Table 1: Intra-Assay-Variation**

	Number of determinations	Mean value [pg/ml]	VC%
Sample 1	6	1028.13	2.6
Sample 2	6	1275.95	4.0
Sample 3	6	1325	5.3

**Table 2: Inter-Assay-Variation**

	Mean value (pg/ml)	Standard deviation	VC%
Sample 1	762	55.3	7.3
Sample 2	1085	52.5	4.8
Sample 3	790	65	8.2

## Recovery

Serum spiking experiments with recombinant human Ghrelin yielded a recovery of 97% (± 2%).

## SPECIMEN COLLECTION, PREPARATION AND STORAGE

**Serum/ EDTA-Plasma** samples are suitable (inappropriate are Heparin- and Citrate-Plasma). An external sample preparation prior to assay is not required.

For testing of single blood samples, the specimens may be taken in the morning or early afternoon. For specific questions, the influence of food intake should be taken in consideration.

Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen at -20°C or below in tightly closable plastic tubes. Avoid on principal repeated freeze-thaw cycles of serum/plasma (if required, please

subaliquote) although Ghrelin levels were found to be unaffected by few cycles (5x) in our experiments.

Because of the wide effective range of this RIA kit a preparative sample dilution is generally not necessary. For most of the determinations (serum or plasma samples, and no extreme values expected) **the use of undiluted samples 100 µl per tube**, should be appropriate. In case of extremely high Ghrelin levels the sample should and can be diluted in Assay Buffer AB, e.g. 1:10. At a concentration of 1000pg/ml the precision of this assay is maximal.

## REAGENTS PROVIDED

1)	<b>CAL</b> 1-6	<b>Standards STD 1-6</b> , lyophilized, <b>750 µl</b> , contain human recombinant Ghrelin. The calibration curve covers a range of <b>2-6.4 ng/ml</b> Ghrelin. Please use <b>100 µl</b> standard solution <b>per tube</b> .
2)	<b>NSB</b>	<b>unspecific binding, NSB</b> , lyophilized, <b>1ml</b> . rabbit IgG
3)	<b>BUF</b> AB	<b>Assay Buffer AB</b> , ready-to-use , <b>30 ml</b> , for reconstitution of <b>1.AB, TR, NSB, STD</b> and <b>CS</b>
4)	<b>BUF</b> PR	<b>Precipitation-Reagent PR</b> , after addition of 2.Ab ready-to use, dilution of 2.Ab 1:56, <b>55ml</b>
5)	<b>1.Ab</b>	<b>1. antibody, 1.Ab</b> , lyophilized, <b>10,5 ml</b> , Reconstitute in 10.5 ml AB.
6)	<b>2.Ab</b>	<b>2. antibody, 2.Ab</b> , lyophilized, <b>1 ml</b> , anti-rabbit-IgG. Reconstitute in 1 ml AB. <b>2.Ab</b> Reconstitute with <b>1 ml</b> reagent <b>AB</b> . Transfer dissolved material to reagent <b>PR</b> immediately before use (Ratio 1:56). Please mix only the required quantity of <b>PR+2.Ab</b> (500µl/vial). The rest can be frozen. The assay is unaffected by the possible occurrence of turbidity after adding <b>2.Ab</b> to reagent <b>PR</b> .
7)	<b>Tracer</b>	<b>Tracer TR</b> , lyophilized, <b>10.5 ml</b> , < 2.3 µCi or < 85 kBq. reconstitute in 10.5 ml AB, stained red
8)	<b>Control</b>	<b>Control CS</b> , lyophilized, <b>750 µl</b> , reconstitute in 750 µl AB. Concentration is given by label +/- 2SD.

## MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes (100 and 200µl) Micropipettes and multichannel pipettes with disposable plastic tips  
 Disposable polystyrene or polypropylene tubes. Conical tubes are highly recommended because of the small volume of the immunoprecipitates

Vortex-mixer

Centrifuge

Device to aspirate the fluid from the tubes (recommended because of the potential danger of radioactivity and infection by human samples)

Ice-Cold deionized water

Gamma Counter

## REAGENT PREPARATION

In conducting the assay, follow strictly the test protocol. Room temperature incubation means: Incubation at 20 - 25°C.

Reagents with different lot numbers should not be mixed. All reagents are stable unopened until the expiry date, if stored in the dark at 2° - 8°C (see label).

**Control Serum** CS Reagent 1.Ab, 2.Ab, TR, NSB and STD have to be reconstituted in **Assay Buffer AB**. It is recommended to keep the reconstituted reagents at room temperature for 30 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer. Transfer reconstituted 2.Ab into the buffer PR. The assay is unaffected by the possible occurrence of turbidity after add in 2.Ab to reagent PR.

The shelf life of the components after opening is not affected, if used appropriately. Reconstituted Components should be stored at -20°C (or below). Repeated freeze-thaw cycles have to be avoided.

Before use, all kit components should be brought to room temperature, if nothing different is indicated. **Precipitates, possible in buffers, should be dissolved before use through mixing and warming.**

## WARNINGS AND PRECAUTIONS

**For research and professional use only.**

Possession and use of the kit is subject to the regulations of the national nuclear regulatory authorities.

Reagents with different lot numbers should not be mixed.

Reagents contain Sodium-Azide as preservative, however, highly diluted (0.02%). Sodium-Azide is very toxic, R-Phrases: 28, 32, 50/53 and S-Phrases 28, 45, 60, 61 must be considered.

Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**, if not indicated differently. Precipitates in buffers should be dissolved before use by thorough mixing and warming. **Temperature WILL affect** the assay. However, values for the samples will not be affected.

Caution: This kit contains material of human and/or animal origin. Source human serum for the Control Serum provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibodies. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and US specimens should be treated as potentially infectious.

**Radioactivity** - Before ordering or using radioactive materials, it is necessary to take the appropriate actions to ensure compliance with national regulations governing their use. Local rules in each establishment, which define actions and behaviour in the radioactivity working areas, should also be adhered to. The advice given here does not replace any local rules, instructions or training in the establishment, or advice from the radiation protection advisers. It is important to follow the code of good laboratory practice in addition to the specific precautions relating to the radionuclide I-125 used.

Iodine-125 has a radioactive half-life T<sub>1/2</sub> of 60 days and emits 35.5 keV gamma radiation, 27 – 32 keV x-rays and no beta radiation. Shielding is effectively done by lead, first half value layer is 0.02 mm lead, reduction to 10 % is made by 0.2 mm.

To reduce the radiation dose time spent handling radioactivity should be minimized (plan ahead), and distance from source of radiation should be maximized (doubling the distance from the source quarters the radiation dose).

Formation of aerosols, e.g. by improper opening and mixing of vials or pipetting of solutions which may cause minute droplets of radioactivity become airborne, is a hazard and should be avoided.

Solutions containing iodine should not be made acidic, because this might lead to the formation of volatile elemental iodine.

As some iodo-compounds can penetrate rubber gloves, it is advisable to wear two pairs, or polyethylene gloves over rubber.

For cleaning of contaminated areas or equipment, the Iodine-125 should be rendered chemically stable by using alkaline sodium thiosulphate solution together with paper or cellulose tissue.

#### **General first aid procedures:**

Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

#### **The handling of radioactive and potentially infectious material must comply with the following guidelines:**

The material should be stored and used in a special designated area.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Avoid direct contact with these materials by wearing laboratory coats and disposable gloves.

Spilled material must be wiped off immediately. Clean contaminated areas and equipment with a suitable detergent.

Unused radioactive material and radioactive waste should be disposed according to the recommendations of the national regulatory authorities.

## ASSAY PROCEDURE

NOTES: All determinations (Standards, Control Serum and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the Standards, Control Serum and the samples should be pipette as fast as possible.

### Flow Chart of Assay Protocol

Tube Nr. :	Contents	AB	STD, CS, Samples	NSB	1.Ab	TR	2.Ab in PR
1,2	Total Counts	-	-	-	-	100	-
3,4	NSB	100	-	100	-	100	500
5,6	B <sub>0</sub> (zero standard)	100	-	-	100	100	500
7-18	Standards 1-6	-	100 STD1-6	-	100	100	500
19,20	Control	-	100 CS	-	100	100	500
21,22	Sample 1	-	100	-	100	100	500
23,24	Sample 2	-	100	-	100	100	500
etc.							

All volumes are given in µl.

- 1 ) Labelling of the assay tubes should be done in the following order (duplicates):
  - 1, 2 total counts (**TC**),
  - 3, 4 non specific binding (**NSB**)
  - 5, 6 zero standard (**B<sub>0</sub>**),
  - 7-18 **Standards** 1 to 6
  - 19, 20 Control **CS**
  - 21,22 etc. **samples**.
- 2 ) Add **100 µl** of reagent **AB** (Assay Buffer) to tubes 3, 4 and 5,6.
- 3 ) Add **100 µl** of **Standards 1-6** to tubes 7-18:
  - 7, 8 Standard 1 (200 pg/ml)
  - 9, 10 Standard 2 (400 pg /ml); etc. up to 18.
- 4 ) Add **100 µl** Control **CS** to tubes 19, 20.
- 5 ) Add **100 µl** of **sample** to tubes 21, 22, etc.
- 6 ) Add **100 µl NSB** to tubes 3 and 4.
- 7 ) Add **100 µl** of Reagent **1.Ab** (1<sup>st</sup>. Antibody), beginning with tube 5.
- 8 ) Mix tubes with a Vortex-Mixer and incubate **overnight** at 2-8°C (**at least 20 h, maximal 24 h**).
- 9 ) Add **100 µl** of Reagent **TR** (Tracer) to all tubes.  
**Seal tubes 1 and 2 (total counts) with a stopper and remove until step 16.**
- 10 ) Mix the remaining tubes with a Vortex-Mixer and **incubate overnight (at least 16 h, maximal 20 h) at 2-8°C**.
- 11 ) Add from Reagent **2.Ab** to **PR** (1:56, mix); **the reagent mix must be cooled (2-8 °C)**.  
Add **500 µl** Reagent **PR** (incl. 2.Ab), beginning with tube 3.
- 12 ) Mix tubes with a Vortex-Mixer and incubate for precipitation for **1 h at 2-8°C**.
- 13 ) Add **1 ml ice-cold water**.
- 14 ) Centrifuge at 2-4°C at **3000 x g for 20 min**.
- 15 ) Aspirate the supernatant. In order not to destroy or aspirate the small precipitate a rest of approx. 2 mm supernatant should be left over the precipitate. (Tip: Add limit stop to the aspirate needle). Depending on laboratory equipments and common laboratory practice supernatant can also be decanted carefully.
- 16 ) Count the radioactivity of all tubes in Gamma-Counter for **1 to 3 min**.

### **Extended washing procedure for increased precision:**

After the Incubation of 1 hour (step 12) centrifuge the tubes (s. step 14) and aspirate the supernatant (s. step 15). Add directly 1 ml ice-cold water. This should not be done too vigorously in order to keep the precipitate intact. **Do not mix again!** Centrifuge the tubes once again at 3000g for 5 min, aspirate the supernatant and count the radioactivity of all tubes in the gamma-counter.

This extended procedure results in a somewhat higher precision bound up with higher work expenditure. The higher precision may be relevant only in special cases.

## **CALCULATION OF RESULTS**

### **Establishing the Standard Curve**

Standard	B <sub>0</sub>	1	2	3	4	5	6
ng/ml	0	0.2	0.4	0.8	1.6	3.2	6.4
pg/ml	0	200	400	800	1600	3200	6400
pmol/l	0	59	119	237	475	949	1899

1. Calculate the average counts of each pair of tubes.
2. Subtract the average of NSB (NSB, Tubes 3 and 4) from the mean counts of the Standards, controls and samples. This corresponds to the corrected B values.
3. The corrected value of the zero standard (tubes 5 and 6) equals B<sub>0</sub>.
4. Calculate the percent bound (%B/B<sub>0</sub>):  $\%B/B_0 = B/B_0 \times 100 \%$
5. Plot %B/B<sub>0</sub> versus the standard concentrations on a semi-logarithmic or logit-log paper respectively or per computer analysis.
6. For quality control calculate the percentage of %NSB/TC:  
*NSB / TC (average counts of tubes 3 and 4 / average counts of tubes 1 and 2) x 100 %.* It should be: %NSB/TC < 5%
7. Quality control, Calculate the %B<sub>0</sub>/TC:  
*B<sub>0</sub> (see step 3.) / TC (total counts) x 100 %.*  
It should be: %B<sub>0</sub>/TC > 20%

### **Evaluation of sample concentrations:**

Read the Ghrelin concentration value (abscissa) corresponding to the %B/B<sub>0</sub>-Wert of the sample as in the example given below:

Example:

Average counts of the tubes 3 and 4 (NSB): 482 cpm  
Average counts of the tubes 5 and 6 (Zero standard, B<sub>0</sub>): 8927 cpm  
Average counts of the tubes 19 and 20 (Control CS): 4794 cpm

$$\%B/B_0 = \frac{\text{Sample} - \text{NSB}}{B_0 - \text{NSB}} \times 100\% = \frac{4794 - 482}{8927 - 482} \times 100\% = 51.1\%$$

For a 51.1% value on the y-axis (ordinate) corresponds in this example abscissa value of 1055 pg/ml.

Multiplication of this value determined graphically or by aid of a computer program with the dilution factor gives the ghrelin concentration of the sample.

If it is preferred to express the results as pmol/l, the values given as pg/ml have to be divided by 3.371 to obtain pmol/l.

Example: 1055 pg/ml : 3.371 = 313 pmol/l.

**Concentration of the control:**

The control CS should fit with the labelled concentration range. The measured Ghrelin concentrations are only valid, if the measured value of the control is in the labelled concentration range. Otherwise process analysis is required and depending on the results the measured values are accepted or not.

**Exemplary Values**

Concentration of Ghrelin in human sera varied from minimal 300 pg/ml (90 pmol/L) to maximal 4000 pg/ml (1200 pmol/L) so far. Whereas in most of the samples the concentration ranged between 600 pg/ml (180 pmol/L) and 1400 pg/ml (420 pmol/L).

52 sera or plasma from each healthy male and female blood donors, age 20 to 65 years, were measured regarding their Ghrelin concentration with Mediagnost Ghrelin RIA R90. However, there is no information available regarding the nutritional status of the blood donors. The results in pg/ml are presented in the table 3.

**Table 3: Ghrelin serum concentration of male and female blood donors.**

	<b>Male</b>	<b>Female</b>
<b>n</b>	52	52
<b>Mean</b>	<b>801.3</b>	<b>1141.9</b>
<b>Standard Deviation</b>	179.1	570.9
<b>Min</b>	511.0	454.4
<b>Max</b>	1,555.2	2,565.7
<b>Median</b>	770.5	1,032.3

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## SUMMARY – Mediagnost Ghrelin R90

Reconstitution of the Reagents		
<b>1<sup>st</sup>Antibody (1.Ab)</b>	in Assay Buffer (AB)	10.5 ml
<b>Tracer (TR)</b>	in Assay Buffer (AB)	10.5 ml
<b>NSB</b>	in Assay Buffer (AB)	1 ml
<b>Standards (1-6)</b>	in Assay Buffer (AB)	750 µl
<b>Control serum CS</b>	In Assay Buffer (AB)	750 µl
<b>2<sup>nd</sup>Antibody (2.Ab)</b>	in Assay Buffer (AB) Mix solution with PR. Mix only the required quantity of PR+2 <sup>nd</sup> Ab	1 ml (1 ml 2.Ab+ 55 ml PR) or Ratio (1:56)

Serum or plasma **samples** can be used **undiluted**.

Double Determinations		Addition of Reagents [µl]			
Tube Nr. :	Contents	AB	STD (1-6), CS, Samples	NSB	1.AB (1 <sup>st</sup> Antibody)
1,2	Total Counts	-	-	-	-
3,4	NSB	100	-	100	-
5,6	B <sub>0</sub> (zero standard)	100	-	-	100
7-18	Standards (1-6)	-	100 <b>STD (1-6)</b>	-	100
19,20	Control serum (CS)	-	100 <b>CS</b>	-	100
21,22	Sample 1	-	100	-	100
23,24	Sample 2	-	100	-	100
etc.					

**Mix** all tubes with a Vortex-mixer.

**Incubation overnight (at least 20 h, maximal 24 h) at 2-8°C**

Add **100 µl** Reagent **TR** (Tracer) to all tubes.

**Seal the tubes 1 and 2 (total counts)** with a stopper and **remove until the step 16**.

**Mix** the remaining tubes with a Vortex-Mixer.

**Incubation overnight (at least 16 h, maximal 20 h) at 2-8°C**

Add from the Reagent **2.Ab to PR (1:56, mix)**, the reagent mix must be cooled (**2-8°C**)  
Add **500 µl** reagent **PR** (inc. 2.Ab.), beginning with the tube 3.

**Mix** tubes with a Vortex mixer.

**Incubation 1 h at 2-8°C**

Add **1 ml ice-cold water**, beginning with the tube 3.

**Centrifugation at 2-4°C, □3000 x g, 20 min**

**Aspirate** the supernatant  
(a rest of approx. 2 mm of supernatant should be left over the intact precipitate).

**Count the radioactivity** of all the tubes in a Gamma-Counter.



<b>CAL</b> 1-6	STD 1-6	Rec in 750 µl AB	
<b>Control</b>	CS	Rec in 750 µl AB	
<b>NSB</b>	NSB	Rec in 1 ml AB	
<b>2.Ab</b>	2.Ab	Rec in 1 ml AB	<b>DILU</b> PR 1:56 °C 2- 8
<b>1.Ab</b>	1. Ab	Rec in 10.5 ml AB	
<b>Tracer</b>	TR	Rec in 10.5 ml AB	
<b>SPE</b>			100 µl

°C 20-25 °C

<b>Tubes</b>			<b>BUF</b> AB	<b>CAL</b>	<b>NSB</b>	<b>1.Ab</b> 1.Ab
1/2	<b>Tracer</b> TR = <b>TC</b>	-	-	-	-	-
3/4	<b>NSB</b>	-	100 µl	-	100 µl	-
5/6	<b>B<sub>0</sub></b>	-	100 µl	-	-	100 µl
7/8	<b>CAL</b> STD 1 (0,2 ng/ml)	-	-	100 µl	-	100 µl
9/10	<b>CAL</b> STD 2 (0,4 ng/ml)	-	-	100 µl	-	100 µl
11/12	<b>CAL</b> STD 3 (0,8 ng/ml)	-	-	100 µl	-	100 µl
13/14	<b>CAL</b> STD 4 (1,6 ng/ml)	-	-	100 µl	-	100 µl
15/16	<b>CAL</b> STD 5 (3,2 ng/ml)	-	-	100 µl	-	100 µl
17/18	<b>CAL</b> STD 6 (6,4 ng/ml)	-	-	100 µl	-	100 µl
19/20	<b>CONTROL</b> KS	100 µl	-	-	-	100 µl
21/22	<b>SPE</b>	100 µl	-	-	-	100 µl

↔

🕒 20-24h °C 2- 8

100µl **Tracer** TR ↔

🕒 16-20h °C 2- 8

500µl **2.Ab** **DILU** PR 1:56 °C 2- 8 **ALL** **NOT** **TC** ↔

🕒 1 h °C 2- 8

1 ml A. dest °C 2- 8 **ALL** **NOT** **TC**

🌀 ≥ 3000× g 20 min °C 2-8

**ASP** **ALL** **NOT** **TC****MEASURE**