

ENG

Product Data Sheet:

**HUMAN GROWTH HORMONE  
BINDING PROTEIN (GHBP)  
ELISA**

Catalogue number:  
**RMEE024R**

**For research use only!**

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## 1. INTENDED USE

For quantitative detection of Human GHBP in serum and plasma sample.

- For research and professional use only!
- Sandwich Enzyme Immunoassay
- Incubation time: 3 hours
- Antibodies: specific, high-affinity polyclonal antibodies
- Buffer: ready to use
- Reference material: eukaryotic, recombinant GHBP
- Standard: 6 single standards: 0.05 – 4ng/mL, recombinant GHBP
- Assay Range: 0.006 – 84 ng/mL
- Control: 2 control sera, freeze-dried - RiliBäK conform
- Sample: human serum/plasma
- Sample volume: 15 µL
- Sample dilution: 1:21
- Analytical Sensitivity: 0.006 ng/mL
- Intra/Interassay Variance: <10 / <10 %

Example Version

## 2. INTRODUCTION

Growth Hormone Binding Protein (GHBP) consists of 238 amino acids and includes four sites for glycosylation and three disulfide bonds. In humans GHBP is formed by receptor shedding of the growth hormone receptor by a metalloprotease (ADAM17).

In equilibrium about 50% of circulating growth hormone (GH) is bound to GHBP but only 2% of the circulating GHBP bound a GH molecule with a stoichiometry of 1:1. Only in case of supraphysiological GHBP levels a 2:1 ratio appears. The complex of GH and GHBP has an approximate molecular weight of 80 kDa (GHBP 60 kDa). In an animal model (guinea pig) the complex formation increases half-life from 11-20 minutes up to about 100 minutes and in general binding to GHBP inhibits GH cellular action.

### GHBP Physiology

GHBP concentration is independent of GH pulsatility and does not show a circadian rhythm. GHBP levels are low until 2-6 months of life, increase steeply in the first two years and continue to increase slowly until early adulthood. From the 4th decade the GHBP serum concentration declines slowly.

GHBP correlates positively with the intraabdominal fat mass and is increased in type II diabetics with hyperinsulinaemia. It is not known whether the tight relationship between fat mass and circulating GHBP results from GHBP expression in adipocytes or any other mechanism.

From a diagnostic point of view undetectable GHBP levels could point to a GH insensitivity, caused by a deletion in the GH-receptor gene. Further, the IGF-I/GHBP ratio might be an indicator for GH-deficiency in adults, in particular in women. It could also be predictive for GH treatment response.

The strong positive relationship with intraabdominal fat mass might be a hint, that GHBP is a possible biomarker for the amount of visceral adipose tissue.

### 3. ASSAY PRINCIPLE

The Biovendor assay is based on polyclonal antibodies and recombinant GHBP, expressed in eukaryotic cells.

The Biovendor ELISA for GHBP RMEE024R is a so-called Sandwich-Assay. It utilizes two specific antibodies of high affinity. First the GHBP in the sample binds to the immobilized antibody on the microtiter plate. In a two-step sequence, the biotin-conjugated anti-GHBP-Antibody and the streptavidin-peroxidase are bound. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the GHBP content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

The Biovendor GHBP ELISA, RMEE024R allows secure and reproducible measurement of GHBP in human body fluids and is a suitable tool for the investigation of GHBP as biomarker in energy and fat metabolism. In a preliminary study GHBP was measured in serum of healthy blood donors and mean concentration of 16.28 ng/mL was detected (Range: 12.48 -22.31).

Example Version

#### 4. REAGENTS PROVIDED

The reagents listed below are sufficient for 96 wells including the standard curve

<b>MTP</b>	<b>Microtiter plate</b> , ready for use, coated with rabbit-anti-GHBP-antibody. Wells are separately breakable.	<b>(8x12) wells</b>
<b>A-F</b>	<b>Standards</b> , lyophilized, (recombinant GHBP in rabbit serum), concentrations are given on vial labels and on the QC-certificate.	<b>6x 750 µL</b>
<b>KS1</b>	<b>Control Serum 1</b> , lyophilised, (human serum), concentration is given on the QC-certificate.	<b>1 x 250 µL</b>
<b>KS2</b>	<b>Control Serum 2</b> , lyophilised, (human serum), concentration is given on the QC-certificate.	<b>1 x 250 µL</b>
<b>AK</b>	<b>Antibody-Conjugate</b> , ready for use, contains rabbit biotinylated anti-GHBP antibody.	<b>1 x 12 mL</b>
<b>EK</b>	<b>Enzyme-Conjugate</b> , ready for use, contains Streptavidin-Peroxidase Conjugate.	<b>1 x 12 mL</b>
<b>PP</b>	<b>Sample Buffer</b> , ready for use, <b>Please shake before use!</b>	<b>1 x 120 mL</b>
<b>WP</b>	<b>Washing Buffer</b> , 20-fold concentrated solution	<b>1 x 50 mL</b>
<b>S</b>	<b>Substrate</b> , ready for use, horseradish-peroxidase-(HRP) substrate, stabilised H <sub>2</sub> O <sub>2</sub> Tetramethylbencidine.	<b>1 x 12 mL</b>
<b>SL</b>	<b>Stopping Solution</b> , ready for use, 0.2 M sulphuric acid.	<b>1 x 12 mL</b>
-	<b>Sealing Tape</b> , for covering the <b>microtiter plate</b> .	<b>3 x</b>

## 5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer WP (A. dest.), 950 mL
- Vortex-mixer
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Micro plate reader ("ELISA-Reader") with filter for 450 and  $\geq 590$  nm
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)

## 6. WARNINGS AND PRECAUTIONS

The BioVendor kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol.

Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. BioVendor will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

**Caution: This kit contains material of human and/or animal origin. Therefore all components and patient's specimens should be treated as potentially infectious.**

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

### Human Serum

Following components contain human serum: **Control Sera KS1, KS2**

Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

### Reagents AK, EK, PP, WP

Contain as preservatives f **5-chloro-2-methyl-4-isothiazolin-3-one** and **2-methyl-4-isothiazolin-3-one** (<0.015%)

- |           |   |
|-----------|---|
| H317      | May cause an allergic skin reaction.  |
| P280      | Wear protective gloves/ protective clothing/ eye protection/ face protection. |
| P272      | Contaminated work clothing should not be allowed out of the workplace.        |
| P261      | Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.                        |
| P333+P313 | If skin irritation or rash occurs: Get medical advice/ attention.             |
| P302+P352 | IF ON SKIN: Wash with plenty of soap and water.                               |

P501 Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

### Substrate Solution (S)

The TMB-Substrate (S) contains 3,3',5,5' Tetramethylbencidine (<0.05%)

- H315 Causes skin irritation.  
 H319 Causes serious eye irritation.  
 H335 May cause respiratory irritation.  
 P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.  
 P305+P351+ IF IN EYES: Rinse cautiously with water for several minutes.  
 P338 Remove contact lenses, if present and easy to do. Continue rinsing.

### Stopping Solution (SL)

The Stopping solution contains 0.2 M acid sulphur acid (H<sub>2</sub>SO<sub>4</sub>)

- H290 May be corrosive to metals.  
 H314 Causes severe skin burns and eye damage.  
 P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.  
 P301+P330+ IF SWALLOWED: rinse mouth.  
 P331 Do NOT induce vomiting.  
 P305+P351+ IF IN EYES: Rinse cautiously with water for several minutes.  
 P338 Remove contact lenses, if present and easy to do. Continue rinsing.  
 P309+P310 IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

### General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove 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: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

## 7. SAMPLE

### Serum and Plasma

Serum and Heparin/EDTA plasma yield comparable values.

The GHBP levels are reduced in citrate plasma samples, because of the relatively high amount of anticoagulant.

### 7.1 Speciment Collection

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided.

### 7.2 Required Sample Volume

15  $\mu$ L

### 7.3 Required Sample Volume

In firmly closable sample vials

- Storage at 4°C: max. 3 days
- Freezer /-thaw cycles: max. 3

Freeze-thaw cycles should be minimized. Up to 3 cycles showed no effect on the measured GHBP concentration.

First experiments with native serum samples were conducted, incubating these samples at 20-25°C and 27°C for three days. A significant decay of GHBP was detected in the samples incubated at 37°C (>20%). The decrease in GHBP at ambient temperature was less prominent (-8 to -13%) and at 4°C no significant change was detected.

### 7.4 Interference

Neither triglycerides, bilirubin nor hemoglobin exert any influence up to concentrations of 100 g/L, 200 mg/L, 5 g/L respectively on the measurement of GHBP in human serum.

### 7.5 Sample Dilution

- **Dilution:** 1: 21 with sample buffer PP
- **Example:** 15  $\mu$ L sample to 300  $\mu$ L sample buffer PP provided (21 dilution factor)

### 7.6 GHBP in healthy adults

Exemplary GHBP was measured in healthy human blood donors (n=10). The mean GHBP concentration detected was 16.28ng/mL (Range: 12.5 to 22.3)

## 8. TECHNICAL RECOMMENDATIONS

### Storage Conditions

Store the kit at 2-8°C after receipt until its expiry date. The lyophilized reagents should be stored at -20 °C after reconstitution. Avoid repeated thawing and freezing.

### Storage Life

The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The reconstituted components standards **A-F** and Control Sera **KS1** and **KS2** must be stored at -20°C (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay. The 1:20 diluted Washing Buffer **WP** is 4 weeks stable at 2-8°C

### Preparation of reagents

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.

### Reconstitution

The Standards **A – F** and Controls **KS1** and **KS2** are reconstituted with the Sample Buffer **PP**. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

### Dilution

After reconstitution dilute the Control Sera **KS1** and **KS2** with the Sample Buffer **PP** in the same ratio (1:21) as the sample.

The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

### Assay Procedure

When performing the assay, Blank, Standards **A-E**, Control Serum **KS1** and **KS2** and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody-HRP-Conjugate **AK** as well as the succeeding Substrate Solution **S** should be added to the plate in the same order and in the same time interval as the samples. Stopping Solution **SL** should be added to the plate in the same order as Substrate Solution **S**.

All determinations (Blank, Standards **A-E**, Control Sera **KS1** and **KS2** and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

## Incubation

Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate Solution **S**, stabilised H<sub>2</sub>O<sub>2</sub>-Tetramethylbencidine, is photosensitive—store and incubation in the dark.

## Shaking

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must be adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/ or false values, excessive shaking may result in high optical densities and/ or false values.

## Washing

Proper washing is of basic **importance** for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided Washing Buffer **WP** diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

The danger of handling with potentially infectious material must be taken into account.

When using an **automatic microtiter plate washer**, the respective instructions for use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

**Manual washing** is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

## 9. ASSAY PROCEDURE

Preparation of reagents		Reconstitution:	Dilution
A-F	Standards	in 750 µL Sample Buffer PP	-
KS1	Control Serum 1	in 250 µL Sample Buffer PP	1:21 with PP
KS2	Control Serum 2	in 250 µL Sample Buffer PP	1:21 with PP
WP	Washing Buffer	-	1:20 with Aqua dest.
Sample dilution: with Sample Buffer PP 1:21. <b>Don't use samples undiluted!</b>			
<b>Before assay procedure bring all reagents to room temperature 20-25°C.</b>			
Assay Procedure in Double Determination:			
Pipette	Reagents	Position	
100 µL	Sample Buffer PP as Blank	A1/A2	
100 µL	Standard A (0.05 ng/mL)	B1/B2	
100 µL	Standard B (0.1 ng/mL)	C1/C2	
100 µL	Standard C (0.5 ng/mL)	D1/D2	
100 µL	Standard D (1 ng/mL)	E1/E2	
100 µL	Standard E (1.5 ng/mL)	F1/F2	
100 µL	Standard F (4 ng/mL)	G1/G2	
100 µL	Control Serum KS 1 (1:21 diluted)	H1/H2	
100 µL	Control Serum KS 2 (1:21 diluted)	A3/A4	
100 µL	Sample (1:21 diluted)	in the rest of the wells according the requirements	
Cover the wells with the sealing tape.			
<b>Sample Incubation: 1 h at 20-25°C, 350 rpm</b>			
5 x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well	In each well	
100 µL	Antibody -Conjugate AK	In each well	
Cover the wells with the sealing tape.			
<b>Incubation: 1 hour at 20-25°C, 350 rpm</b>			
5 x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well	In each well	
100 µL	Enzyme -Conjugate EK	In each well	
Cover the wells with the sealing tape.			
<b>Incubation: 30 minutes at 20-25°C, 350 rpm</b>			
5 x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well	In each well	
100 µL	Substrate Solution S	In each well	
<b>Incubation: 30 Minutes in the Dark at 20-25°C</b>			
100 µL	Stopping Solution SL	In each well	
	Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.		

## 10. QUALITY CONTROL

GLP requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. All kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated.

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of standard F should be above 1.00.

Samples, which yield higher absorbance values than Standard F, should be re-tested with a higher dilution.

## 11. EVALUATION OF RESULTS

### 11.1 Establishing the standard curve

The standards provided contain the following concentrations of GHBP:

Standard	A	B	C	D	E	F
ng/mL	0.05	0.1	0.5	1	1.5	4

1. Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).
2. Subtract the mean absorbance of the blank from the mean absorbances of all other samples and standards.
3. Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4. Recommendation: Calculation of the standard curve should be done by using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. **A higher-grade polynomial, or four parametric logistic (4-PL) curve fit or non-linear regression are usually suitable for the evaluation** (as might be spline or point-to-point alignment in individual cases).
5. The GHBP concentration in ng/mL (or pg/mL, according the chosen unit for the standards) of the samples can be calculated by **multiplication** with the respective **dilution factor** Quality Criteria

## 11.2 Example of Typical Standard Curve

The exemplary data and the standard curve in Figure 1 cannot be used for the calculation of the test results. You have to establish a standard curve for each test you conduct.

Table 1: Data of typical Standard Curve

Standard	A	B	C	D	E	E
ng/mL	0.05	0.1	0.5	1	1.5	4
OD <sub>450-620</sub>	0.055	0.105	0.470	0.924	1.294	2.909

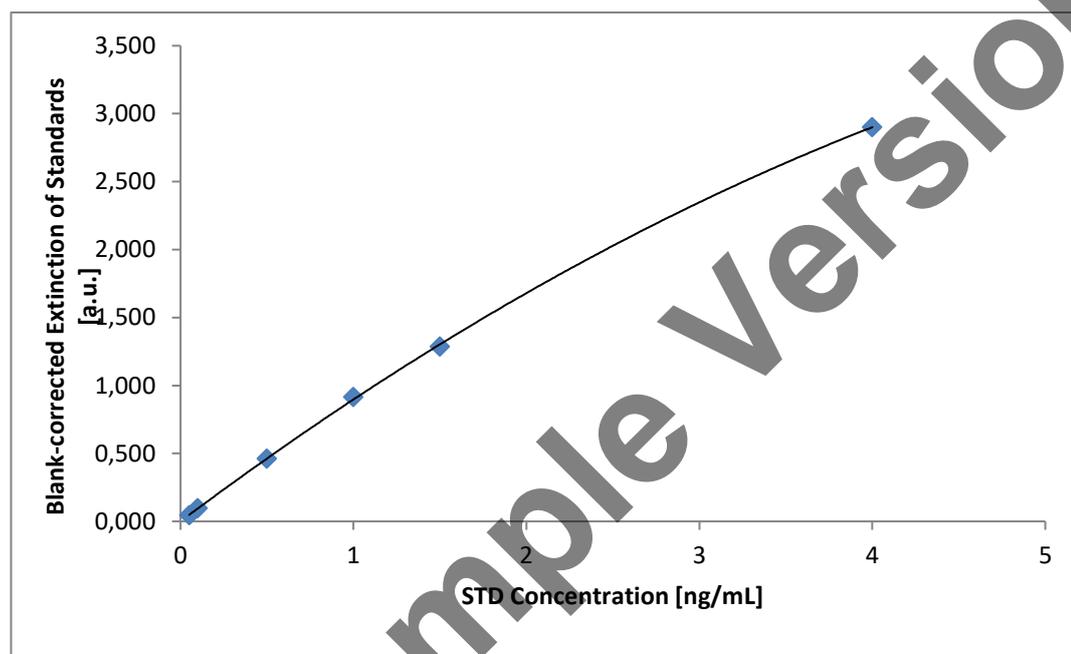


Figure 1: Exemplary Standard Curve

### 11.3 Evaluation of sample concentration

Sample dilution: 1:21

Measured extinction of your sample: 0.624

Measured extinction of the blank: 0.059

Your measurement program will calculate the GHBP concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit. In this exemplary case the following equation is solved by the program to calculate the GHBP concentration in the sample:

$$0.565 = -0.0592x^2 + 0.964x$$

$$X = 0.609 \text{ ng/mL}$$

If the dilution factor (1:21) is taken into account the GHBP concentration of the undiluted sample is:

$$0.609 \text{ ng/mL} \times 21 = 12.798 \text{ ng/mL} / \mu\text{g/L}$$

## 12. LIMITATION OF PROCEDURE

The Biovondor GHBP ELISA RMEE024R is based on polyclonal antibodies. The measurement results determined by this technique can be influenced by heterophilic antibodies. The potential influence of these antibodies was minimized by assay design but can never be excluded completely. Further, several physiological substances like triglycerides were tested regarding their influence on GHBP measurement and no significant influence was detected. But in theory there might be other substances or other concentration which interfere with GHBP measurement.

## 13. PERFORMANCE CHARACTERISTICS

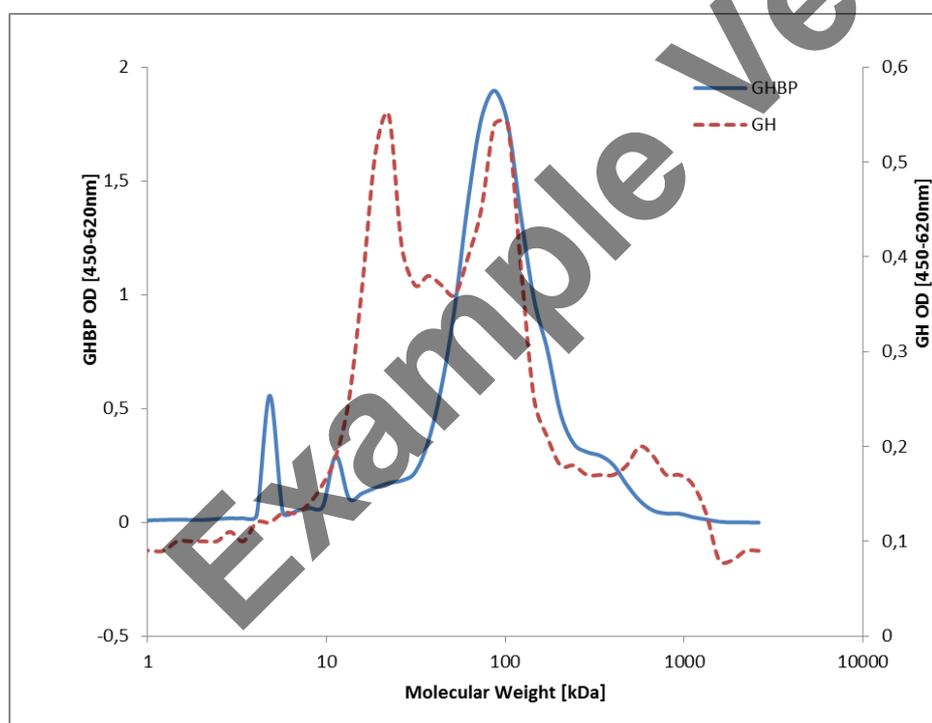
### 13.1 Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the Biovendor RMEE024R is 0.006 ng/mL as mean, in 3 independent determinations values ranging from 0.007 to 0.005 ng/mL were found.

Sensitivity was assessed further by determination of serially dilutions of native human serum samples (see Linearity). In these experiments samples were diluted up to 1:320 and GHBP measured in the dilution was 0.03 ng/mL. Theoretically, defined as the 10 standard deviations of the blank the limit of quantification is also 0.03 ng/mL GHBP.

### 13.2 Specificity

The specificity of the antibodies used for GHBP detection in the Biovendor GHBP ELISA RMEE024R was evaluated by size exclusion chromatography analysis of human serum enriched with recombinant growth hormone (Fig 2) and subsequent analysis of SEC fractions by Biovendor E022 or GHBP antibodies.



**Figure 2** SEC analysis of a human serum enriched with growth hormone

A human serum was enriched with 3 µg/mL recombinant growth hormone (GH). After an incubation period of >20 h at 4°C, the enriched serum was separated by size exclusion chromatography (Superdex 10/300; Flow rate: 0.5 ml/min; Fraction size: 0.4 mL/min). GHBP and GH were measured after dilution in Biovendor Dilution buffer by Biovendor GHBP-Antiserum and Biovendor GH ELISA E022.

The analysis reveals that GHBP antibodies detect a signal at 100 kDa and a small shoulder could be interpreted as an additional signal at 340 kDa. As expected GH is detected at 22 kDa (unbound GH), at 100kDa and a second peak appears >340 kDa. This shows that the protein detected by

GHBP antibodies binds GH, which indicates a GH-binding property of the detected protein. The expected molecular weight of a GH/GHBP complex is 80 kDa, slightly less than the size of the GH/GHBP complex the Biovendor antibodies detect. This is probably caused by methodological variability or differential glycosylation of native GHBP.

### 13.3 Recovery

1 ng/mL recombinant GHBP was added to human serum. The GHBP content of the so enriched samples was measured and recovery calculated. Results are shown in table 2.

**Table 2: Recovery of recombinant human GHBP in serum**

[µg/L]	Sample 1	Sample 2	Sample 3
Sample	17.73	16.61	10.07
1 ng/mL + GHBP	18.81	17.28	12.51
% Recovery	100	98	113

### 13.4 Precision

#### Intra-Assay-Variation

A native serum sample has been measured 16 times on different positions on the plate and at a mean concentration of 14.89 ng/mL GHBP (SD 0.652) an intra-assay variability of 4.38% was detected.

#### Inter-Assay-Variation

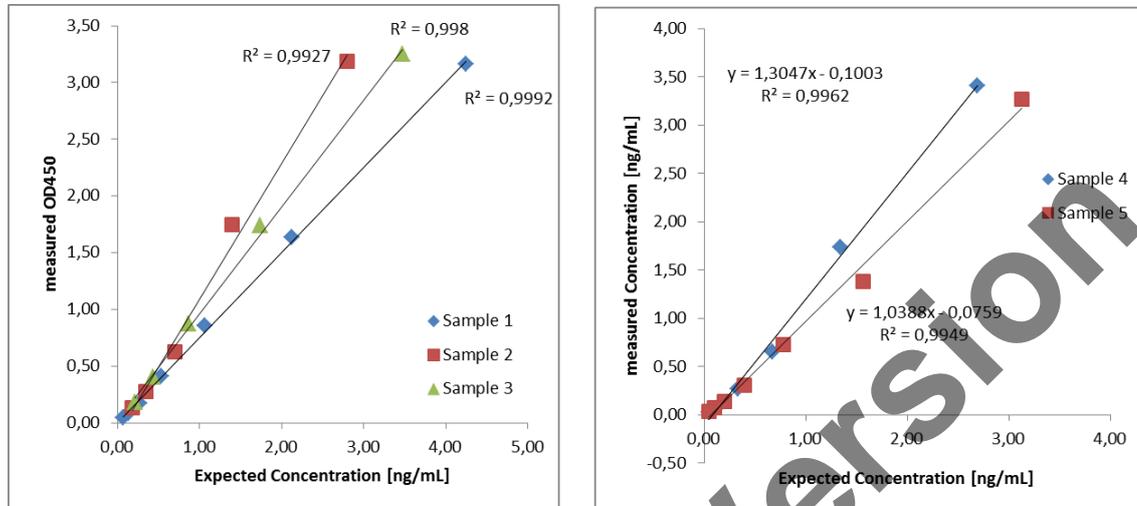
Serum samples were measured in independent assays. On average the coefficient of variation was 7.72% (Range 3.08– 10.67%). Exemplary results are shown in table 3.

**Table 3: Inter-Assay-Variation**

	Number of measurements	Mean value [ng/mL]	Standard Deviation [ng/mL]	VC (%)
Sample 1	7	12.61	0.39	3.08
Sample 2	8	21.21	1.8	8.47
Sample 3	8	15.66	1.02	6.5

### 13.5 Linearity

Linearity was tested by dilution of native sera with different GHBP contents (Sample 1-5). The optical density was measured and plotted against the expected GHBP concentration. Linearity was analysed by linear regression, a coefficient of correlation  $>0.9$  indicates a good linearity (Figure 3).



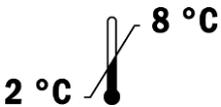
**Figure 3 Linearity:** Several samples were diluted in sample buffer from 1:5 up to 1:320 and the absolute signal or the recalculated concentration are shown in comparison to the expected concentration.

A closer look to the data revealed that a dilution of 1:10 is possible but good linearity is realized from a dilution of 1:20 in sample buffer. Here the deviation of the mean is less than 30%.

## 14. INTERNATIONAL ASSAY DESCRIPTION

A-F	STD	Rec in	750 µL	BUF	PP	-
KS1	Control	Rec in	250 µL	BUF	PP	1:21 DILU BUF PP
KS2	Control	Rec in	250 µL	BUF	PP	1:21 DILU BUF PP
WP	WASHBUF	20x	-			1:20 DILU A. dest.
-	SPE					1:21 DILU BUF PP
-	°C	20-25 °C				
100 µL	BUF	PP				A1/A2
100 µL	STD	A	(0.05 ng/mL)			B1/B2
100 µL	STD	B	(0.1 ng/mL)			C1/C2
100 µL	STD	C	(0.5 ng/mL)			D1/D2
100 µL	STD	D	(1.0 ng/mL)			E1/E2
100 µL	STD	E	(1.5 ng/mL)			F1/F2
100 µL	STD	F	(4 ng/mL)			G1/G2
100 µL	CONTROL	KS1	1:21	DILU	BUF	PP H1/H2
100 µL	CONTROL	KS2	1:21	DILU	BUF	PP A3/A4
100 µL	SPE	1:21	DILU	BUF	PP	
	TAPE					
<b>1 h / 20-25 °C / 350 rpm</b>						
5x 300 µL	5x	WASHBUF	WP			
100 µL	Ab	AK				
	TAPE					
<b>1 h / 20-25°C / 350 rpm</b>						
5x 300 µL	5x	WASHBUF	WP			
100 µL	CONJ	EK				
	TAPE					
<b>0.5 h / 20-25°C / 350 rpm</b>						
5x 300 µL	5x	WASHBUF	WP			
100 µL	SUBST	TMB	S			
<b>0.5 h / 20-25°C</b> ▷						
	H <sub>2</sub> SO <sub>4</sub>	SL				
	MEASURE					

## 15. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
 <a href="http://www.biovendor.com">www.biovendor.com</a>	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks





BioVendor – Laboratorní medicína a.s.  
Karásek 1767/1, 621 00 Brno, Czech Republic  
+420 549 124 185  
[info@biovendor.com](mailto:info@biovendor.com)  
[sales@biovendor.com](mailto:sales@biovendor.com)  
[www.biovendor.com](http://www.biovendor.com)

Example Version

