Human Growth Hormone Binding Protein (GHBP) ELISA E024

Growth Hormone Binding Protein (GHBP) consists of 238 amino acids and includes four sides for glycosylation and three disulfide bounds. 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 (guniea 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.

Physiological functions: Regulation of biological availability of GH is possibly the physiologic role of GHBP. Thus, the half-life of GH is prolonged up to 100 minutes by binding to GHBP.

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.

Clinical Relevance
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 intra-abdominal fat mass might be a hint, that GHBP is a possible biomarker for the amount of visceral adipose tissue.

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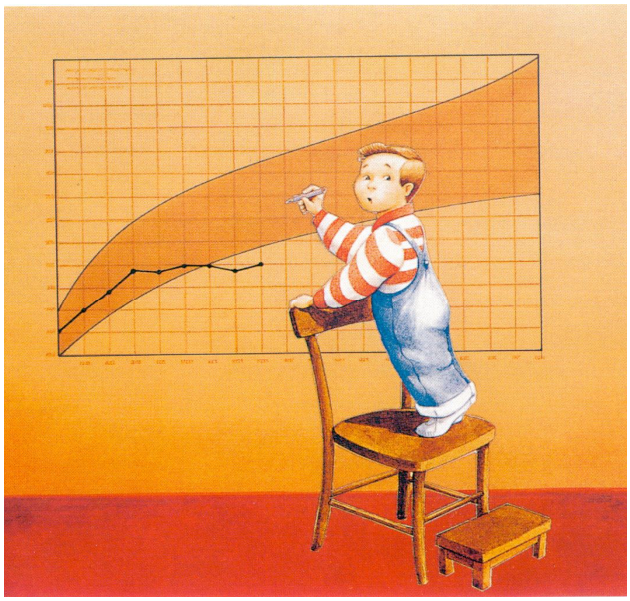
Fisker S Physiology and pathophysiology of growth hormone-binding protein: Methodological and clinical aspects. Growth Hormone & IGF Research 2006: 16 (1-28)

Mediagnost
Growth Hormone Binding Protein Assay (GHBP)

The Mediagnost GHBP ELISA, E024 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).

Recombinant human GHBP, produced in eukaryotic cell lines, is used for assay standardization.



Assay Features E024

- ü Analytical sensitivity of **0.006 ng/mL**
- ü Results on your hands in about 3 h
- ü Standard material, recombinant GHBP 0.05, 0.1, 0.5, 1.0, 1.5, 4 ng/mL
- ü Intra- and Inter-assay variance < 10%
- ü Recovery in human serum 98-113%
- ü 2 internal control sera: RiliBÄK conform

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SUMMARY OF THE ASSAY PROCEDURE E024

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. Don´t use samples undiluted!**

Before assay procedure bring all reagents to room temperature **20-25°C**.

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.

Sample Incubation: 1 h at 20-25°C, 350 rpm		
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.		
Incubation: 1 hour at 20-25°C, 350 rpm		
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.		
Incubation: 30 minutes at 20-25°C, 350 rpm		
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
Incubation: 30 Minutes in the Dark at 20-25°C		
100 µL	Stopping Solution **SL**	In each well
	Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.	

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