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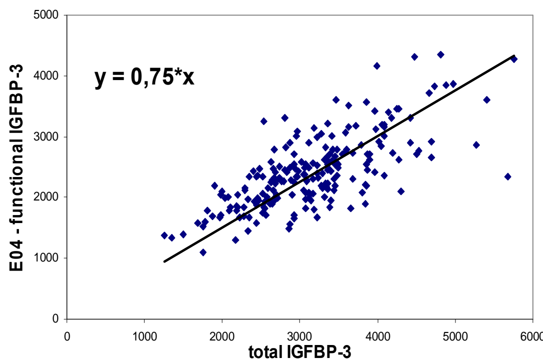
Gesellschaft für Forschung und Herstellung von Diagnostika GmbH
Quality made in Germany

functional
Insulin-like Growth Factor Binding Protein-3 LIA E04A*

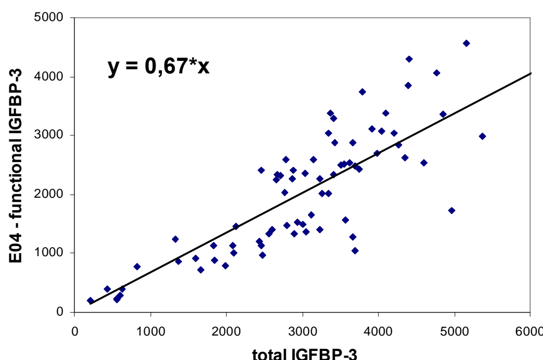
The main physiological function of IGF-Binding Proteins is the regulation of bioavailability of IGF-I and –II. IGF-I forms a ternary complex with the acid-labile subunit and IGFBP-3 from which it can be released selectively by **proteolytical cleavage**. Therefore the cleavage products of the proteolysis also appear in serum besides the intact IGFBP-3.

Do you know what you are measuring?

The plot of total-IGFBP-3 values against functional IGFBP-3 shows that **25 %** of the determined total-IGFBP-3 is not functional.

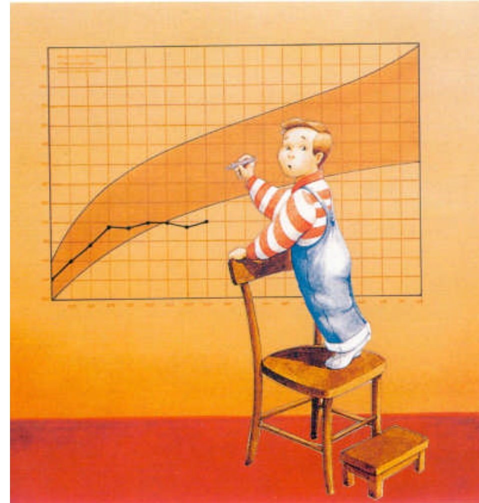


The part of intact IGFBP-3 is reduced by e.g. **tumor- and liver diseases** as well as by **growth disorders.**



The proteolysis of IGFBP-3 is changed during several physiological conditions:

- **Tumors**
- **Catabolic States**
- **Burns**
- **Pregnancy**



A clear gender difference was obvious in the relative part of functional IGFBP-3 by comparison of healthy **men and women**:

	Total IGFBP-3; min/max(ng/ml)	Funct. IGFBP-3; min/max(ng/ml)	Functional IGFBP-3 (% of total)
females	3568; 1752/5752	2506; 1102/4346	70,2
males	2752; 1258/4726	2332; 1337/4304	84,8
overall	3160	2419	77,5

Assay - Characteristics
Performance like ELISA Protocol – very fast & simple

- ✓ small sample requirement, thus ideal for **paediatric patients**
- ✓ no cross reactions
- ✓ direct correlation to total-IGFBP-3 (the same sample dilution can be used)
- ✓ IGF-I binding IGFBP-3 is detected only
- ✓ analytical sensitivity of 0.18 ng/ml
- ✓ wide linear range
- ✓ 5 single standards (0.4 -30 ng/ml))
- ✓ Intra-Assay Variance < 5,6%
- ✓ Inter-Assay Variance < 6,8%
- ✓ rapid assay procedure: **Incubation 3.5h**

*** Patent pending DE19719001**

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Mediagnost functional IGFBP-3 LIA E04A

Reconstitution/ Dilution of Reagents:		
Standards A-E	Reconstitution in **Sample Buffer PP** (green)	**1 ml each**
Control Seren KS1 & KS2	Reconstitution in **Sample Buffer PP** (green)	**250 µl**
Ligand Conjugate LK **Enzyme Conjugate EK**	Dilute LK and EK 1:101 each in **Dilution Buffer VP** (e.g. **60 µl LK** plus **6 ml** VP each) (e.g. **120 µl EK** plus **12 ml** VP each)	**1:101 each**
Washing Buffer WP	dilute in **A. dest.** (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with A.dest. to 1000 ml)	**1:20**
Sample Dilution + Control Sera KS1 & KS2: 1:505 in Sample Buffer PP (green colored), **mix directly and use within max. 60 min.** Use **50 µl per determination** (pipetting control= blue coloration)		
Before assay procedure bring all **reagents** to **room temperature**		

Proposal of Assay Procedure for Double Determination:

Pipette	Reagents	Well Positions
50 µl	**diluted Ligand Conjugate** LK	Pipette **in all required number of wells**
50 µl	Sample Buffer **PP** as Blank	A1 and A2
50 µl	Standard **A** (**0.4 ng/ml**)	B1 and B2
50 µl	Standard **B** (**2 ng/ml**)	C1 and C2
50 µl	Standard **C** (**6 ng/ml**)	D1 and D2
50 µl	Standard **D** (**15 ng/ml**)	E1 and E2
50 µl	Standard **E** (**30 ng/ml**)	F1 and F2
50 µl	diluted Control Serum **KS1**	G1 and G2
50 µl	diluted Control Serum **KS2**	H1 and H2
50 µl	**diluted Samples**	Pipette samples in the rest of the wells according to requirements
Cover the wells with the sealing tape		

Incubation: 2 h **at RT,** 350 rpm

5x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP**/ **well**	each well
100 µl	diluted Enzyme Conjugate **EK**	each well

Incubation: 1 h at RT, 350 rpm

5x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP**/ **well**	each well
100 µl	Substrate Solution **S**	each well

Incubation: 30 min **in** the dark **at RT**

100 µl	Stop Solution **SL**	each well
Measure the absorbance within 30 min at **450 nm** (≥590 nm Reference)		