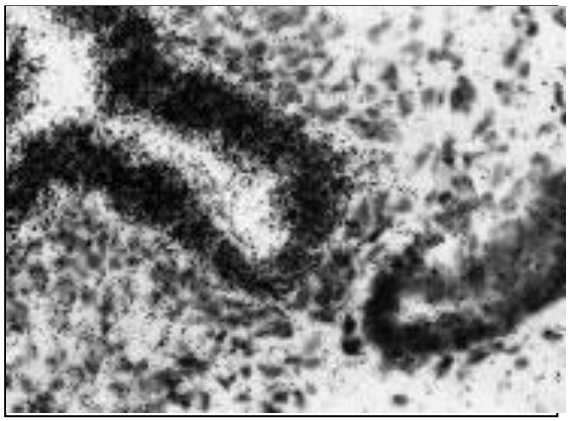
Insulin-like Growth Factor Binding Protein-1 ELISA E01

Insulin-like growth factors (IGF)-I and -II are bound to specific binding proteins (IGFBPs) in the circulation. Today the existence of at least six binding proteins and several related proteins has been shown.

IGFBP-1 contains 234 amino acids, with a predicted molecular mass of 25 kDa. The human IGFBP-1 gene is located on chromosome 7. The major sites of IGFBP-1 synthesis are fetal and adult liver and decidualized endometrium.

Serum levels of IGFBP-1, which reflect its synthesis by the liver, exhibit considerable diurnal variation. Circulating IGFBP-1 levels are highest early in the morning, lowest in the evening. The levels are high in the fetus and newborn, but decline steadily until puberty. The mean level in **healthy adults is 5.01 ng/ml (range 0.23-17.94 ng/ml).** After about 65 years of age, serum IGFBP-1 levels begin to increase. There is also an inverse correlation between body mass index (BMI) and fasting serum IGFBP-1 concentrations.



Uterine IGFBP-1 Expression

The most important regulator of circulating IGFBP-1 is insulin. Fasting insulin and IGFBP-1 concentrations are inversely correlated. During a 3-h glucose tolerance test there is a decrease of about 50 % in serum IGFBP-1 levels. A meal also has a decreasing effect.

Aging is associated with **decreased** suppression of serum IGFBP-1 by insulin. In insulin-dependent diabetes (IDDM), serum IGFBP-1 levels are high. In non-insulin-dependent diabetes, in which insulin levels are high, serum IGFBP-1 is low. Low levels are also seen in acromegaly, Cushing's disease, and PCO (polycystic ovarian syndrome).

Changes in **endometrial IGFBP-1 production are not reflected in serum levels**, indicating that endometrial IGFBP-1 production cannot be assessed by serum IGFBP-1 measurement.

In amniotic fluid, IGFBP-1 levels are 100 - to 1000-fold higher than in maternal serum.

In the case of ruptured fetal membranes, IGFBP-1 leaks through the cervix into the vagina. Thus, detection of IGFBP-1 in vaginal secretions at a concentration greater than that in blood can be interpreted as indicating rupture of fetal membranes.

Human IGFBP-1 Assay

A monoclonal antibody specific to human IGFBP-1 is immobilized on microwell plates, and another monoclonal antibody, also specific to IGFBP-1, is conjugated with horse-radish peroxidase (HRP). No cross-reactions have been observed with recombinant IGFBP-2 or IGFBP-3 up to concentrations of 500 ng/mL.
Mediagnost IGFBP-1 ELISA has an **European Approval for Clinical Diagnostics**: EC Directive 98/79EG (CE).

Assay Features IGFBP-1 E01

- ✓ Analytical sensitivity of ø < 0.1 ng/ml
- ✓ Single standards: 0, 0.1, 0.5, 1, 2, 4 and 8 ng/ml, **native human IGFBP-1**
- ✓ Intra- / Inter-Assay Variance ø < 10%
- ✓ 2 control sera included: RiliBÄK conform
- ✓ Linearity has been shown for dilutions 1:5 up to 1:512.

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Mediagnost IGFBP-1 ELISA E01

ASSAY PROCEDURE

Preparation of reagents		**Reconstitution:**	**Dilution**
A-G	**Standards**	in **500 µL** Dilution Buffer **VP**	-
KS1	**Control Serum 1**	in **250 µL** Dilution Buffer **VP**	**≥ 1:16** with **VP**
KS2	**Control Serum 2**	in **250 µL** Dilution Buffer **VP**	**≥ 1:16** with **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**

Sample dilution: with Dilution Buffer **VP ≥ 1:16**

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

Assay Procedure in Double Determination:

Pipette	**Reagents**	**Position**
50 µL	Antibody Conjugate **AK**	Pipette **in <u>all</u> required number of wells**
50 µL	Standard **A** (**0 ng/mL**)	A1/A2
50 µL	Standard **B** (**0.1 ng/mL**)	B1/B2
50 µL	Standard **C** (**0.5 ng/mL**)	C1/C2
50 µL	Standard **D** (**1 ng/mL**)	D1/D2
50 µL	Standard **E** (**2 ng/mL**)	E1/E2
50 µL	Standard **F** (**4 ng/mL**)	F1/F2
50 µL	Standard **G** (**8 ng/mL**)	G1/G2
50 µL	Control Serum **KS 1** (≥ 1:16 diluted)	H1/G2
50 µL	Control Serum **KS 2** (≥ 1:16 diluted)	A3/A4
50 µL	Sample (≥1:16 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation <u>with Shaking</u>: 1 h at 20°C - 25°C, <u>350 rpm</u>		
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 <u>with Shaking</u>: 30 Minutes at 20°C - 25°C, <u>350 rpm</u>		
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: 15 Minutes in the Dark at 20°C - 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.		