Mouse- / Rat-Insulin-like Growth Factor Binding Protein 3 ELISA E031

Growth Hormone, Insulin-like Growth Factors and their binding proteins build up an endocrine system regulating not only longitudinal growth in humans but also influencing a broad variety of other physiological and pathophysiological processes like energy metabolism or tumor growth.

Most effects of Growth Hormone (GH) are exerted by Insulin-like Growth Factors (IGF) mainly produced by the liver but also locally by specific tissues.

The effects of IGF are also regulated. Specific binding proteins (IGFBP 1-7) regulate bioavailability of IGF. After proteolytic cleavage of the binding proteins IGF is set free and able to bind to its receptor.

The autophosphorylation of this thyrosine kinase receptor activates intracellular signalling cascades. Some of these IGFBPs not only regulate the availability of IGF but also exert IGF-independent effects on cell physiology.

IGFBP-3 is the most abundant IGFBP in circulation and therefore of special relevance in regulation of IGF effects.

This is reflected by the indicative value of serum IGFBP-3 concentration in diagnostics of **growth disturbances**. IGFBP-3 has also been shown to be able to **induce apoptosis, promote tumor growth and inhibit cellular migration and metastasis** dependent on tissue and tumor stage.

This enzyme immunoassay kit is suited for measuring **IGFBP-3 in mouse and rat specimens** like serum, cell lysates.

The Mediagnost ELISA for mouse-/ rat-IGFBP-3 (m/rIGFBP-3) E031 is a so-called Sandwich-Assay.

The IGFBP-3 in the sample binds to the immobilized antibody on the microtiter plate. In the following step, the biotinylated and Streptavidin-Peroxidase conjugated specific anti-mouse IGFBP-3-Antibody binds in turn to the immobilised IGFBP-3.

The peroxidase will convert the dye and the resulting colour intensity is depending on the concentration of m/rIGFBP-3 in the samples.

Assay Characteristics
Mediagnost MOUSE- / RAT-IGFBP-3 E031

- ✓ recombinant mouse IGFBP-3 Standard 0.39 - 25 ng/mL
- ✓ Analytical sensitivity of 0.09 ng/mL
- ✓ High Precision Inter-/ Intra-Assay Variance of ø < 10%
- ✓ Low cross-reactivity to recombinant human eukaryotic expressed IGFBP-3
- ✓ High recovery of recombinant rodent IGFBP-3 92.6% in DMEM + 5% FCS
- ✓ Linearity given for sample dilutions of 1:100 to 1:3200
- ✓ Low sample volume: recommended dilution of 1:505 results in 10 µL sample volume

Mediagnost Gesellschaft für Forschung und Herstellung von Diagnostika GmbH Aspenhaustr 25 72770 Reutlingen Deutschland
+49 7121 51 4840 - www.mediagnost.de - contact@mediagnost.de

Mediagnost mouse/rat IGFBP-3 ELISA E031

ASSAY PROCEDURE

Reagent preparation		**Reconstitution**	**Dilution**
A-G	**Standards**	in **750 µL** Dilution Buffer **VP**	-
KS1	**Control Serum 1**	in **250 µL** Dilution Buffer **VP**	**1:505** with Dilution Buffer **VP**
KS2	**Control Serum 2**	in **250 µL** Dilution Buffer **VP**	**1:505** with Dilution Buffer **VP**
WP	**Waschpuffer**	-	**1:20** with **Aqua dest.**

Dilute **Samples** with Dilution Buffer **VP 1:505**

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

Assay Procedure in Double Determination:

Pipette	**Reagents**		**Position**
100 µL	Dilution Buffer **VP** (Blank)		A1/A2
100 µL	Standard **A** (**0.39 ng/mL**)		B1/B2
100 µL	Standard **B** (**0.78 ng/mL**)		C1/C2
100 µL	Standard **C** (**1.56 ng/mL**)		D1/D2
100 µL	Standard **D** (**3.13 ng/mL**)		E1/E2
100 µL	Standard **E** (**6.25 ng/mL**)		F1/F2
100 µL	Standard **F** (**12.5 ng/mL**)		G1/G2
100 µL	Standard **G** (**25 ng/mL**)		H1/H2
100 µL	Control Serum **KS 1**	(1:505 diluted)	A3/A4
100 µL	Control Serum **KS 2**	(1:505 diluted)	B3/B4
100 µL	Sample	(1:505 diluted)	In the rest of the wells according to the requirements.
Cover the wells with the sealing tape.			
Incubation: 1 h at 20-25°C, 350 rpm			
5x 300 µL	Aspirate the contents of the wells and wash **5x** with **300 µL** each **WP**/**well**		Each well
100 µL	Antibody Conjugat **AK**		Each well
Cover the wells with the sealing tape			
Incubation: 1 h bei 20-25°C, 350 rpm			
5x 300 µL	Aspirate the contents of the wells and wash **5x** with **300 µL** each **WP**/**well**		Each well
100 µL	Enzyme Conjugate **EK**		Each well
Cover the wells with the sealing tape			
Incubation: 15 min at 20-25°C, 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
Substrat S Incubation: 15 Minutes in the dark at RT			
100 µL	Stop Solution **SL**		Each well
Measure the absorbance within 30 min at **450 nm** (≥590 nm Reference)			