Version 10-04/24

User's Manual

Mouse/Rat IGFBP-3 ELISA Kit

REF **DEIA4628**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This ELISA kit is suited for measuring IGFBP-3 in mouse and rat serum and plasma and in cell culture medium.

General Description

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 intra cellular 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. Regulation is effected e.g. through nourishing situation; Different diets for example affect the IGFBP-3 concentration. 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.

Mouse/rat models for in vivo experiments are often used for studies of IGF-dependent and independent effects of IGFBP-3, particularly in the field of tumor research. For this purpose CD offers the product as a reliable and sensitive test system for the determination of IGFBP-3 in mouse and rat samples.

Principles of Testing

The CD Mouse/Rat IGFBP-3 ELISA Kit is a sandwich-assay. It utilizes two different specific high affinity polyclonal antibodies for this protein. The IGFBP-3 in the samples binds quantitatively to the immobilized antibody. In the following step, the biotinylated antibody in turn binds IGFBP-3. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antibody. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the IGFBP-3 content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

Reagents And Materials Provided

1. **MTP**, Microtiter plate(96 wells), ready for use, coated with goat anti-mouse IGFBP-3 Antibody, wells are separately breakable.
2. **A-G**, Standards (7×750 µL), lyophilised (native Mouse-IGFBP-3), Concentrations are given on the vial labels and quality certificate.
3. **KS1**, Control Serum 1 (1×250 µL), lyophilised, (Mouse Serum), Concentration is given **on the quality**

certificate.

4. **KS2**, Kontrollserum 2 (1×250 µL), lyophilisiert, (Ratten Serum), Concentration is given **on the quality certificate**.
5. **AK**, Antibody Conjugate (1×12 mL), ready for use, **Goat anti-mouse-IGFBP-3-Antibody, biotinylated**.
6. **EK**, Enzyme Conjugate EK (1×12 mL), contains HRP (Horseradish-Peroxidase)-labeled Streptavidin.
7. **VP**, Dilution Buffer (1×125 mL), ready for use. **Please shake before use**.
8. **WP** , Washing Buffer WP(1×50 mL), 20-fold concentrated solution
9. **S**, Substrate S (1×12 mL), ready for use, horseradish-peroxidase (HRP)-substrate, stabilised Tetramethylbencidine.
10. **SL**, Stopping Solution SL (1×12 mL), ready for use, 0.2 M sulphuric acid.
11. Sealing Tape for covering the microtiter plate (3×)
12. Instructions for use
13. Quality Control Certificate (QC-Certificate)

Materials Required But Not Supplied

1. Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer, 950 mL.
2. Graduated cylinder for diluting Washing Buffer
3. Precision pipettes and multichannel pipettes with disposable plastic tips
4. Polyethylene PE/Polypropylene PP tubes for dilution of samples
5. Vortex-mixer
6. Microtiter plate shaker (350 rpm)
7. Microtiter plate washer (recommended)
8. Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

Storage

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.

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-G 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.

Specimen Collection And Preparation

1. **Sample type**

   Mouse and Rat Serum Plasma, In Heparin-Plasma samples the levels were found approx. 15%

decreased. Further, cell culture medium was found to be suitable.

2. **Specimen collection**

   Haemolytic reactions have to be avoided.

3. **Requested sample volume: 10 µl serum.**

4. **Sample stability**

   a. In firmly closable sample vials

   b. Storage at -20°C: min. 2 years

   c. Freeze/-thaw cycles: max. 3

   It is recommended to store samples as soon as possible at least at 4°C. For any long time storage the sample has to be kept frozen at -20°C.

5. **Sample dilution**

   Samples **must be diluted** prior to measurement. An extraction step is not required.

   a. Dilution: **1:505** with Dilution Buffer **VP**

   We recommend a dilution in 2 steps: Pipette **1 mL** Dilution Buffer **VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **10 µL** Serum- or Plasma (dilution 1:101) and mix each tube immediately. Pipette **100 µL** of this dilution into another PE/PP vessel with **400 µL** of Dilution Buffer **VP** and mix immediately. This results in a final dilution of 1:505. After mixing, use 100 µL per assay in the assay within 1 hour of this solution.

   b. After Mixing use 100 µL per assay in the assay within 1 hour of this solution.

   c. Where required, depending on the expected IGFBP-3-values, the dilution with Dilution Buffer **VP** can be higher or lower.

Reconstitution And Storage

The Standards A-G and Control KS1 and KS2 are reconstituted with the Dilution Buffer VP. 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.

After reconstitution dilute the Control Sera KS1 and KS2 with the Dilution Buffer VP in the same ratio (1:505) as the sample. The required volume of Washing Buffer WP is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest.

Reagent Preparation

Note: 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.

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	**Washing Buffer**	-	**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

Note:

1. When performing the assay, Blank, Standards A-G, Control Serum KS and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate AK, Enzyme Conjugate EK 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-G, Control Serum KS and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
2. **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.
3. **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 fur 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.

Procedure

1. 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 KS1 (1:505 diluted)	A3/A4
100 µL	Control Serum KS2 (1:505 diluted)	B3/B4
100 µL	Sample (1:505 diluted)	In the rest of the wells according to the requirements

2. Cover the wells with the sealing tape.
3. **Incubation: 1 h at 20-25°C, 350 rpm**
4. Aspirate the contents of the wells and wash 5 times with 300 µL **WP** each well.
5. Add 100 µL Antibody Conjugat **AK** in each well.
6. Cover the wells with the sealing tape.
7. **Incubation: 15 min at 20-25°C, 350 rpm**
8. Aspirate the contents of the wells and wash 5 times with 300 µL **WP** each well.
9. Add 100 µL Substrate Solution **S** in each well.
10. **Substrat S Incubation: 15 Minutes in the dark at room temperature.** Incubation at room temperature means: Incubation at 20-25°C. The Substrate Solution **S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive-store and incubation in the dark.
11. Add 100 µL Stop Solution **SL** in each well.
12. Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength

Calculation

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 G should be above 1.00.

Samples, which yield higher absorbance values than **Standard G**, are beyond the standard curve, for reliable determinations such samples should be retested at a higher dilution.

1. Establishing the standard curve

The standards provided contain the following concentration of **mIGFBP-3**:

Standard	A	B	C	D	E	F	G
ng/mL	0.39	0.78	1.56	3.13	6.25	12.5	25

a. Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).

b. Subtract the mean absorbance of the blank from the mean absorbances of all other values.

c. Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.

d. 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).

e. The **IGFBP-3 concentration in ng/mL** of the samples can be **calculated by multiplication with the respective dilution factor**, division by 1000 converts the values in µg/mL or equal mg/Litre (Example: a measured value was 5.760 ng/mL, Sample was 1:505 diluted: 5.760 × 505= 2909 ng/mL, or 2.909 µg/mL equal to 2.909 mg/L)

2. Calculation of sample concentration

Exemplary calculation of the IGFBP-3 concentration of a diluted sample:

OD 450 nm

Measured extinction (mean value) of your sample 0.749

Measured extinction of the blank (mean value) 0.000

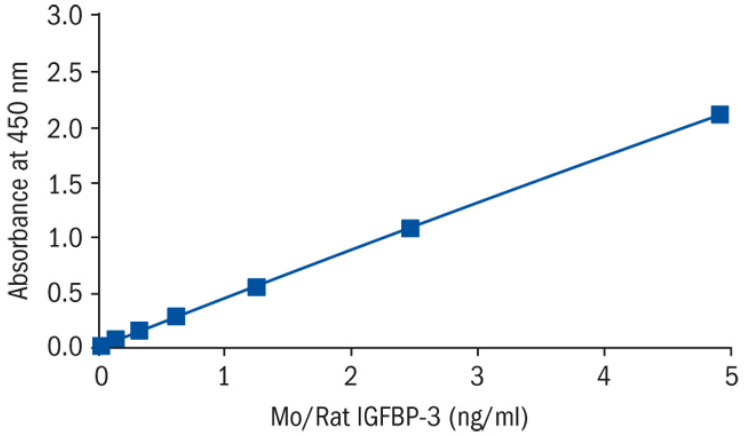
Your **measurement program** will calculate the IGFBP-3 concentration of the sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit (here: polynomial 3rd degree).

In this typical standard curve case the following equation is solved by the program to calculate the IGFBP-3 concentration in the sample:

$$0.749 = 3{,}62^{-6} \times X^3 + -0.00188 \times X^2 + 0.143 \times X - 0.011$$
$$5.760 = X$$

Multiplication by dilution factor (1:505) gives the IGFBP-3 concentration of the sample with **2909 ng/mL**

Typical Standard Curve



Performance Characteristics

Quantitative determination of mouse/rat IGFBP-3 without sample pretreatment

Precision

The Inter- and Intra-Assay variation coefficients were on average ≤10%. Exemplary determinations are shown in table 1 and table 2.

Table 1 Inter-Assay-Variation (n=26 or 15)

	Mean Value (ng/mL)	Standard Deviation (ng/mL)	VC(%)
Sample 1	4836	384	7.94
Sample 2	2625	269	9.43

Table 2 Intra-Assay-Variation (n=13)

	Mean Value (ng/mL)	Standard Deviation (ng/mL)	VC(%)
Sample 1	3286	124	3.76
Sample 2	1529	123	8.02

VC=Coefficient of Variation

Detection Range

0.078-5 ng/ml

Detection Limit

0.018 ng/ml

Sensitivity

The analytical Sensitivity was assessed by 21-fold determination of the blank and calculating the theoretical concentration of the blank +2SD. The analytical sensitivity of the DEIA4628 is 0.09 ng/mL

Specificity

Serum of the cited species were used as diluted samples in this assay system.

No cross reactivity was detected for: Rabbit, Cat, Chicken, Guinea pig, Goat, Sheep, Horse, Donkey, Pig, Dog, Bovine.

Cross reactivity with recombinant human eukaryotic expressed IGFBP-3 (1 µg/mL): 0.06%

Linearity

Linearity (results of 2 different mouse sera)

Dilution	Sample 1 (recalculated, ng/mL)	Sample 2 (recalculated, ng/mL)
1:100	3518	3676
1:200	3691	4145
1:400	3845	4234
1:800	3813	4110
1:1600	3792	4219
1:3200	3861	4557
AV/SD/VC%	3753/129/3.46	4157/284/6.83

AV=Average Value, SD=Standard Deviation; VC=Coefficient of Variation

Recovery

The recovery of recombinant mouse IGFBP-3 in **cell culture medium** DMEM was found to be 89.4%, and, in DMEM incl. 5% FCS 92.6%. Therefore, cell culture medium seems to be suitable as sample matrix.

Precautions

1. **For In Vitro Use only. For Professional use only.** The CD kit is suitable only for in vitro 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. Mediagnost 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. **Animal serum: mouse/rat in the following components: KS1, KS2**

2. Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. Avoid breathing dust/fume/gas/mist/vapours/spray.
3. If skin irritation or rash occurs: Get medical advice/attention. **IF ON SKIN: Wash with plenty of soap and water.**
4. **IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.**
5. **IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.**
6. Dispose of contents/container in accordance with local/regional/national/international regulations.
7. General first aid procedures:

   a. Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.

   b. 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.

   c. Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.