Version 07-11/24

User's Manual

Mouse/Rat IGFBP-2 (insulin-like growth factor binding protein 2) ELISA Kit

REF **DEIA-NB24-13**

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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 enzyme immunoassay kit is suited for measuring IGFBP-2 in mouse and rat serum for scientific purposes.

General Description

Insulin-like growth factors (IGFs) regulate the proliferation, differentation, apoptosis, cell adhesion and metabolism in various tissues and cell types IGFBP-2 is an unglycosylated polypetide of 31.3 kDa, which forms binary IGF-complexes and shows no circadian rhythm in the circulation. The serum concentration of IGFBP-2 increases in fasting, after major surgery and after trauma, but the increasing of the concentration is most intensive in malignant diseases.

The correlation of the IGFBP-2 level to the degree of progression is a striking feature in various tumor types as is the normalization of the IGFBP-serum levels after remission. During the GH-therapy, e.g. in short stature and in GH-abuse (doping) the IGFBP-2 level decreases. In Trisomy 18 IGFBP-2 in maternal serum is decreased and IGFBP-1 is increased; therefore the ratio IGFBP-2 /IGFBP-1 is a marker for this chromosome abnormality.

Transgenic organisms are a good opportunity to investigate the function of genes or proteins. The mouse or rat model is a well-suited system for investigation of the relevance of IGFBP-2 in physiological and pathological processes. Over expression of the IGFBP-2 gene in mice results in a weight reduction of 30% in spleen and moderately reduced weight in other organs. Effects of IGFBP-2 on the organism can be compensated through the modified expression of other IGF Binding proteins.

Especially in tumor biology the mouse and rat systems enable investigation of the systemic relevance of IGFBP-2. IGFBP-2 influences tumor cells as it induces catalase activity in adrenocortical cells. Furthermore IGFBP-2 interacts with tumor cells via its RGD-amino acid sequence and seems to stimulate cell invasion of glioma cells.

Principles of Testing

The Mouse/Rat IGFBP-2 ELISA Kit is a so-called sandwich-assay. It utilizes two different specific high affinity polyclonal antibodies for this protein. The IGFBP-2 in the samples binds quantitatively to the immobilized antibody. In the following step, the biotinylated antibody in turn binds IGFBP-2. 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-2 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. **Microtiter plate:** ready for use, coated with rabbit-anti-IGFBP-2 antibody. Wells are separately breakable. (8x12) wells
2. **Standards (A-G):** lyophilized, (native mouse IGFBP-2), concentrations are given on vial labels and on

quality certificate. 7 x 1 mL

3. **Control Serum (KS):** lyophilized, (mouse serum), concentration is given on quality certificate. 1 x 250 µL
4. **Antibody Conjugate (AK):** 100-fold concentrated, goat-anti-mouse-IGFBP-2 antibody biotinylated. 1 x 120 µL
5. **Enzyme Conjugate (EK):** 100-fold concentrated, Streptavidin Peroxidase-Conjugate. 1 x 120 µL
6. **Dilution Buffer (VP):** ready for use. Please shake before use! 1 x 120 mL
7. **Washing Buffer (WP):** 20-fold concentrated solution. 1 x 50 mL
8. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine. 1 x 12 mL
9. **Stopping Solution (SL):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
10. **Sealing Tape:** for covering the microtiter plate. 2

Materials Required But Not Supplied

1. Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer WP (A. dest.), 950 mL.
2. Precision pipettes and multichannel pipettes with disposable plastic tips
3. Polyethylene PE/Polypropylene PP tubes for dilution of samples
4. Vortex-mixer
5. Microtiter plate shaker (350 rpm)
6. Microtiter plate washer (recommended)
7. Microplate 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 serum KS 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: Serum and Plasma

2. Specimen collection

Haemolytic reactions have to be avoided.

3. Required sample volume: 10 µL

4. Sample stability

In firmly closable sample vials

• Storage at 20-25°C: 3 days

• Storage at 4°C:max 3 Days

• Freeze/-thaw cycles: max.5

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. Samples were kept frozen and freeze-thaw cycles were applied. Up to 5 freeze-thaw cycles did not change the measured IGFBP-2 concentration significantly.

5. Interference

Hemoglobin in the sample do not interfere to a concentration of 5 mg/mL. However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

6. Sample dilution

• Dilution: 1:100 with Dilution Buffer VP

• Pipette 990 µL Dilution Buffer VP in PE-/PP-Tubes add 10 µL Serum.

• If sample size is limiting, a minimum of 2.5 µL sample might be used alternatively, dilution in 250 µL Dilution Buffer VP yields a dilution of 1:101.

• Serum samples should be diluted prior to measurement 1:20 – 1:500-fold with Dilution Buffer VP, depending on the expected values.

Reagent Preparation

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.

1. The Standards A – G is reconstituted with 1 mL Dilution Buffer VP. After resuspension, the standards are diluted according to a gradient - A (31.25 ng/mL), B (62.5 ng/mL), C (125 ng/mL), D (250 ng/mL), E (500 ng/mL), F (1000 ng/mL), G (2000 ng/mL), which are prepared for immediate use.
2. The Control KS is reconstituted with 250 µL Dilution Buffer VP. After reconstitution dilute the Control KS with the Dilution Buffer VP in the same ratio (1:100) as the sample.

Note: 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.

3. The required volume of Washing Buffer WP is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest.

Assay Procedure

Note

1. Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate S, stabilised Tetramethylbencidine, is photosensitive–store and incubation in the dark.
2. 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 S should be added to the plate

in the same order and in the same time interval as the samples. Stop Solution SL should be added to the plate in the same order as Substrate 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.

3. Shaking: The incubation steps should be performed at mean rotation frequency of a microtiter plate shaker. We recommend 350 rpm. Depending on the design of the shaker, the shaking frequency should 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.
4. 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.

   Automatic washing: 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: 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.

Assay Step

1. Set Standards A-G, test samples (1:100 diluted), Control Serum KS (1:100 diluted) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. Aliquot 100ul of Dilution Buffer VP (Blank), Standards A-G, Control Serum KS and test samples into wells.
3. Incubate: Seal the plate with a cover and incubate at 20-25°C, 350 rpm for 1 hour.
4. Wash: Aspirate the contents of the wells, and wash plate 5 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
5. Add 100ul Antibody Conjugate AK (1:100 diluted) into above wells. Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 20-25°C, 350 rpm for 1 hour.
6. Wash: Aspirate the contents of the wells, and wash plate 5 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
7. Add 100ul Enzyme Conjugate EK (1:100 diluted) into above wells. Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 20-25°C, 350 rpm for 30 minutes.
8. Wash: Aspirate the contents of the wells, and wash plate 5 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.

9. Add 100ul of Substrate Solution S into each well, cover the plate and incubate at 20-25°C in dark for 30 minutes.
10. Stop: Add 100ul Stopping Solution SL into each well.
11. Measure the absorbance within 30 min at 450 nm, with ≥ 590 nm as reference wavelength.

Quality Control

Good laboratory practice requires that controls are included in each assay. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state or local standards/laws. The measured control value must be concordant with the valid range stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

Quality criteria

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, should be re-tested with a higher dilution.

Calculation

1. Calculate the mean absorbance value for the blank from the duplicated determination (well A1/A2).
2. Subtract the mean absorbance of the blank from the mean absorbance of all other samples and standards.
3. Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4. 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).
5. The IGFBP-2 concentration in ng/mL (or pg/mL, according the chosen unit for the standards) of the samples can be calculated by multiplication with the respective dilution factor.

Exemplary calculation of IGFBP-2 concentrations

Sample dilution: 1:100

Measured extinction of your sample 0.961

Measured extinction of the blank 0.112

Your measurement program will calculate the IGFBP-2 concentration of the diluted sample automatically. You only have to determine the most suitable curve fit (here: polynomial 2nd degree). In this exemplary case the following equation is solved by the program to calculate the IGFBP-2 concentration in the sample:

$0.849 = -0.0974x^2 + 1.3315x + 0.0024$

$x = 0.673$

If the dilution factor (1:100) is taken into account the IGFBP-2 concentration of the undiluted sample is

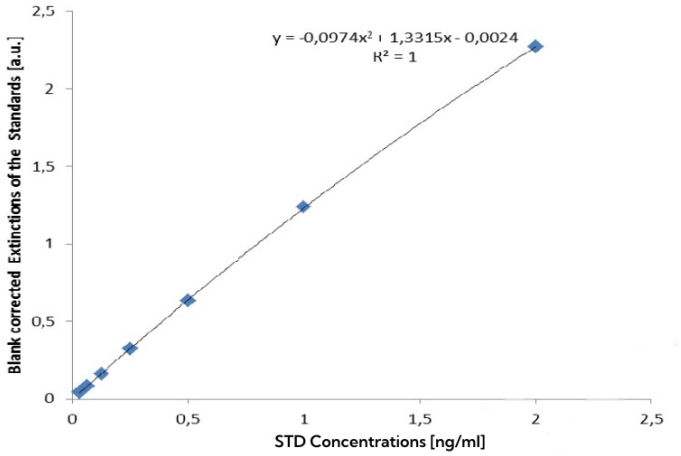
0.673 × 100 = 67.3 ng/mL

Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay.

	Blank	A	B	C	D	E	F	G
pg/mL	0.0	31.25	62.5	125	250	500	1000	2000
$OD_{(450\text{-}620\ nm)}$	0.112	0.042	0.083	0.159	0.325	0.632	1.237	2.27

The exemplary shown standard curve in Figure below cannot be used for calculation of your test results. You have to establish a standard curve for each test you conduct!



Precision

Intra-Assay Variance

Four serum samples where measured in 16 independent assays. On average the coefficient of variation was 5.1% (SD 1.3).

	Determinations [n]	Mean value [ng/mL]	SD	CV [%]
Sample 1	16	124.25	4.08	3.29
Sample 2	16	9.39	0.32	3.41
Sample 3	16	42.22	2.51	5.96

Inter-Assay-Variability

Four serum samples where measured in 16 independent assays. On average the coefficient of variation was 5.1% (SD 1.3).

	Determinations [n]	Mean value [ng/mL]	SD	CV [%]
Sample 1	16	126.07	5.09	4.04
Sample 2	16	102.75	3.95	3.84
Sample 3	16	25.60	1.21	4.73
Sample 4	16	10.40	0.61	5.89

Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank +2SD. The analytical sensitivity of Mouse/Rat IGFBP-2 ELISA Kit is 0.01 ng/ml.

Specificity

The specificity of this ELISA was investigated by testing cross reactivity with human IGFBPs: IGFBP-1, -2, -3, -4, -5, and -6 as well as with recombinant mouse and rat IGFBP-3. These parameters were applied as samples with a concentration of 500 ng/mL. Measured cross reactivity was less than 10% each.

Precautions

1. For research and professional use only. The Creative Diagnostics kit is suitable only for in vitro use and not for internal use in humans and animals.
2. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Creative Diagnostics 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.
3. Do not use obviously damaged or microbial contaminated or spilled material.
4. This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.
5. 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.