Version 07-11/24

User's Manual

Human IGFBP-1 (insulin-like growth factor binding protein 1) ELISA Kit

REF **DEIA-NB24-11**

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.

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PRODUCT INFORMATION

Intended Use

This enzyme immunoassay kit is suited for measuring IGFBP-1 in human serum, plasma or in other body fluids, for example, amnion fluid, mother´s milk, urine or saliva, as for scientific purposes. It is also suited to quantitate IGFBP-1 in cell culture media.

General Description

The Insulin-like Growth Factors I and – II are free in body fluids and tissues but are bound to specific binding proteins. Until today seven different binding proteins (IGFBP-1 to –7) can be differentiated additionally several IGFBP-related proteins have also been detected. Bioavailability of IGF is regulated by these IGFBPs or their proteolytic cleavage which reduces affinity to IGF. The IGFBPs as well as their proteolytic fragments can also exert IGF-independent effects, like influencing cell migration or proliferation.

IGFBP-1 (Placental Protein 12) consists of 234 amino acids and has a molecular weight of approximately 25kDa. The coding DNA region is located on chromosome 7. IGFBP-1 is mainly synthesized by foetal and adult liver tissue and decidual endometrium. Intensity of Expression varies enduring menstruation with a maximal expression in the late secretory phase. Further IGFBP-1 expression seems to be regulated by Insulin concentration, with Insulin inhibiting the expression. Insulin regulation results in diurnal fluctuations of up to factor 10. IGFBP-1 is posttranslational modified by phosphorylation of serine residues 101, 119 and 169. Phosphorylation has physiological relevance as it increases affinity of IGFBP-1 to IGF. In adult humans phosphorylated IGFBP-1 of the liver is the predominant form in circulation. IGFBP-1 produced by endometrial tissue is significantly less phosphorylated than the liver originated form.

In pregnancy IGFBP-1 maternal serum concentration increases significantly with maximal values in the second trimester or 22-23 week of gestation (75.8 ng/ml) and decreases slowly until term. IGFBP-1 concentration is not only increased in maternal but also in foetal serum, extremely high concentrations are found in amniotic fluid. Here concentration can reach more than the 1000-fold of serum values. Long-term changes of serum IGFBP-1 concentration can also be found in amniotic fluid. After birth the IGFBP-1 level of the child decreases until it reaches the low steady-state level of puberty and adulthood.

Short term IGFBP-1 serum concentration is strongly influenced by nutrition level and therewith by insulin. Decreasing IGFBP-1 levels can be found enduring fasting or in diabetes; IGFBP-1 levels increase in case of intensive exercises.

Principles of Testing

The Human IGFBP-1 ELISA Kit is a so-called Sandwich-Assay. It utilizes two specific antibodies of high affinity. First the IGFBP-1 in the sample binds to the immobilized antibody on the microtiter plate. In the following step, the anti-IGFBP-1-Antibody binds in turn to the immobilised IGFBP-1. This is biotinylated and allows the binding of a streptavidin-peroxidase enzyme conjugate. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the IGFBP-1 content of the sample. After the reaction has stopped by the addition of stop solution the color intensity (then yellow) is quantified by measuring the absorption and converted into the IGFBP-1 concentration using a calibration curve.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with mouse-anti-IGFBP-1-antibody. Wells are separately breakable. (8x12) wells
2. **Calibrators (CAL A-G):** lyophilized, (native human IGFBP-1), concentrations are given on vial labels and on quality certificate. 7 x 500 µL
3. **Control 1 (CTR1):** lyophilized, (human serum), concentration is given on quality certificate. 1 x 250 µL
4. **Control 2 (CTR2):** lyophilized, (human serum), concentration is given on quality certificate. 1 x 250 µL
5. **Antibody Conjugate (DET):** ready for use, contains mouse biotinylated anti-hIGFBP-1 antibody. 1 x 6 mL
6. **Enzyme Conjugate (EC):** ready for use, contains HRP (Horseradish-Peroxidase)-labeled Streptavidin. 1 x 12 mL
7. **Dilution Buffer (DIL):** ready for use. Please shake before use! 1 x 125 mL
8. **Washing Buffer (WB):** 20-fold concentrated solution. 1 x 50 mL
9. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine. 1 x 12 mL
10. **Stopping Solution (STP):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
11. **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 Calibrators A-G and Controls CTR1 and CTR2 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

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided. *For blood sampling diurnal variations specially influenced by nutrition should be considered.

3. Required sample volume: 20 µL

4. Sample stability

In firmly closable sample vials

• Storage at 20-25°C: 3 days

• Storage at -20°C: min. 2 years

• Freeze-thaw cycles max. 3

The storage of samples over a period of 2 years at -20°C, showed no influence on the reading. Freezing and thawing of samples should be minimized.

5. Interference

Triglyceride, bilirubin and hemoglobin in the sample do not interfere to a concentration of 100 mg/mL, 100 µg/mL or 5 mg/mL, respectively. However, the use of haemolytic, lipemic or icteric samples should be validated by the user.

6. Sample dilution

• Dilution: ≥1:16 with Dilution Buffer DIL

• Pipette 300 µL Dilution Buffer DIL (red colored) in PE-/PP-Tubes (application of a multi_x0002_stepper is recommended in larger series), add 20 µL Serum- or Plasma (dilution factor 16). After mixing use 50 µL diluted solution within 1 hour per determination in the assay.

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 Calibrators A – G is reconstituted with 500 µL Dilution Buffer DIL. After resuspension, the calibrators are diluted according to a gradient - A (0 ng/mL), B (0.1 ng/mL), C (0.5 ng/mL), D (1 ng/mL), E (2 ng/mL), F (4 ng/mL), G (8 ng/mL), which are prepared for immediate use.
2. The Controls CTR1 and CTR2 are reconstituted with 250 µL Dilution Buffer DIL. After reconstitution dilute the Controls CTR1 and CTR2 with the Dilution Buffer DIL in the same ratio (1:16) 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 WB 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, Calibrators A-G, Control CTR1 and CTR2 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 DET and the Enzyme Conjugate EC 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 STP should be added to the plate in the same order as Substrate S. All determinations (Calibrators A-G, Controls CTR1 and CTR2 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 WB 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 Calibrator A-G, test samples (1:16 diluted), Controls CTR1 and CTR2 (1:16 diluted) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each calibrator and sample in duplicate.
2. Add 50ul Antibody Conjugate DET in all wells used. Aliquot 50ul of Calibrator A-G, Controls CTR1 and CTR2 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 WB. Do not let the wells dry completely at any time.
5. Add 100ul Enzyme Conjugate EC 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.
6. Wash: Aspirate the contents of the wells, and wash plate 5 times with 300 µL Washing Buffer WB. Do not

let the wells dry completely at any time.

7. Add 100ul of Substrate Solution S into each well, cover the plate and incubate at 20-25°C in dark for 30 minutes.
8. Stop: Add 100ul Stopping Solution STP into each well.
9. 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. All calibrators and kit controls must be found within the acceptable ranges as 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 Calibrator A should be below 0.25, and the absorbance of Calibrator G should be above 1.00. Samples, which yield higher absorbance values than Calibrator G, should be re-tested with a higher dilution.

Calculation

1. Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis.
2. Recommendation: Calculation of the calibration 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).
3. The IGFBP-1 concentration in ng/mL of the samples and controls can be calculated by multiplication with the respective dilution factor.

Exemplary calculation of IGFBP-1 concentrations

Sample dilution: 1:16

Measured extinction of your sample 0.199

Your measurement program will calculate the IGFBP-1 concentration of the diluted sample automatically. You only have to determine the most suitable curve fit (here: polynomial 3 rd degree). In this exemplary case the following equation is solved by the program to calculate the IGFBP-1 concentration in the sample:

$$0.199 = -0.00269x^3 + 0.0205x^2 + 0.351x + 0.0097$$

$$x = 0.5235$$

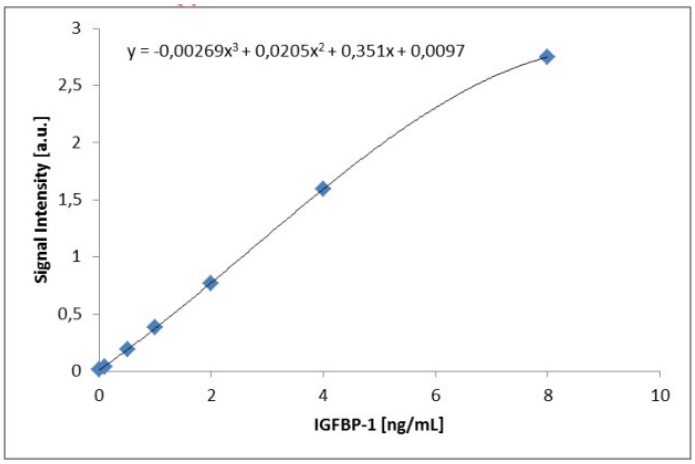
If the dilution factor (1:16) is taken into account the IGFBP-1 concentration of the undiluted sample is

$$0.5235 \text{ ng/mL} \times 16 = 8.376 \text{ ng/mL}$$

Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay. The exemplary shown calibration curve in Figure below cannot be used for calculation of your test results. You have to establish a calibration curve for each test you conduct!

	A	B	C	D	E	F	G
ng/mL	0	0.1	0.5	1	2	4	8
$OD_{(450\text{-}620\ nm)}$	0.012	0.043	0.189	0.381	0.771	1.596	2.748



Precision

Intra-Assay-Variance

Serum samples were measured up to 20 times in the same assay and the coefficient was calculated based on the recalculated IGFBP-1 concentration. The mean measured coefficient of variation (CV) is 6.52% (n =6).

	Determinations [n]	Mean value [ng/mL]	SD	CV [%]
Sample 1	20	4.25	0.24	5.59
Sample 2	20	12.21	0.75	6.11
Sample 3	20	55.17	2.38	4.32

Inter-Assay-Variability

Serum samples were measured in independent assays. On average the coefficient of variation was 6.05% (SD 0.46).

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Mean [ng/mL]	12.50	8.52	6.42	18.62	4.43	9.01
SD	0.17	0.24	0.25	0.52	0.18	0.18
CV [%]	1.33	2.80	3.90	2.80	4.11	2.05
n	5	5	5	5	5	5

Sensitivity

The analytical sensitivity was determined by measuring the null calibration (CAL-A) and calculating the corresponding concentration of the signal intensity of the CAL-A + 2SD. In three different assays analytical

sensitivities from 0.03 to 0.08 ng/mL were measured with an average value of 0.055 ng/mL

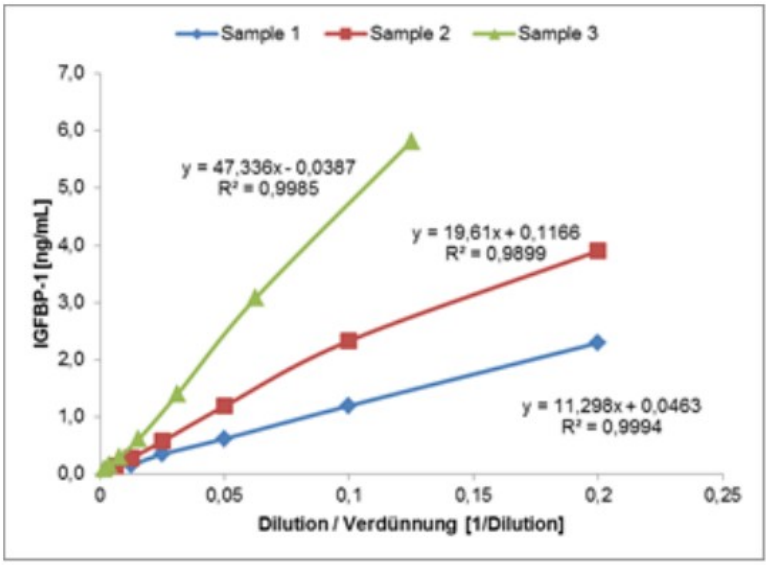
Specificity

The IGF/IGFBP-System consists of several related and homologous proteins. Thus, the crossreactivity of the Human IGFBP-1 ELISA Kit to other IGFBPs was tested exemplarily with the two IGFBPs most abound in circulation.

	Concentration [ng/ml]	Relative cross reactivity [%]
IGFBP-2	0.0071	0.00142
IGFBP-3	0.0033	0.00066

Linearity

Exemplarily three serum sample were diluted 1:5 up to 1:512 and the IGFBP-1 concentration was measured within each dilution. The results were analysed by linear regression analysis and revealed coefficients of determination of >0.98 for each of the samples.



Recovery

Native IGFBP-1 (4.5 ng/mL) was added to human serum and quantified in comparison to the same amount of IGFBP-1 in buffer. The mean recovery detected was 82%. Also, the IGFBP-1 content of samples enriched with recombinant IGFBP-1 was measured and recovery calculated in comparison to enriched buffer (45 ng/mL) with a mean recovery of 94%.

Interferences

Interference of haemoglobin, bilirubin and triglycerides was tested by adding the indicated amount of these substances to human serum containing IGFBP-1. For comparison the same amount of buffer without any

substance was also added to the serum and the relative recovery rate was calculated. Table below shows that on average neither haemoglobin, bilirubin nor triglycerides exert significant influence on the measurement of IGFBP-1 in human serum. But generally, measurement of IGFBP-1 in haemolytic, icteric or lipaemic samples should be avoided.

	Triglyceride (100 mg/mL)	Bilirubin (100 µg/mL)	Hemoglobin (5 mg/mL)
Sample 1	142	72	122
Sample 2	125	46	119
Sample 3	82	113	97

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.
6. Following components contain human serum: Controls CTR1 / CTR2, Calibrators A-G.

   Source human serum for the Controls provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient´s specimens should be treated as potentially infectious.

Limitations

The Human IGFBP-1 ELISA Kit is based on murine antibodies. Generally, this technique is sensible to heterophilic antibodies as well as to human anti mouse antibodies in the sample. The influence of these antibodies is reduced by assay design, but cannot be excluded completely. Further, for interpretation of IGFBP-1 concentrations diagnostic sensitivity and specificity must be taken into account. Also test duration has to be considered, this test system is not suitable for point-of care.