Version 13-11/24

User's Manual

Human IGFBP-2 (insulin-like growth factor binding protein 2) ELISA Kit

REF **DEIA-NB24-12**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This enzyme immunoassay kit is suited for measuring IGFBP-2 in human serum, plasma or in other body fluids, for example, cerebrospinal fluid, breast milk, amniotic fluid, saliva, as for scientific purposes. It is also suited to quantitate IGFBP-2 in cell culture media.

General Description

Insulin-like growth factors (IGFs) regulate the proliferation, differentation, apoptose, cell adhesion and metabolism in various tissues and cell types. The IGF-I, which is produced mainly in liver under the influence of growth hormone (GH), regulates as hormone the linear growth of the bones and the process of sexual maturity, while IGF-II is mainly a growth factor of fetal tissue. The biological actions of IGF over the IGF-Type-I receptor are modulated variably through the IGF binding proteins (IGFBP-1 to-6). IGFBP-2 is, after IGFBP-3, the second most frequent IGFBP in the human blood. IGFs, especially tumor typical pro-IGF-forms and hormones regulate the expression of IGFBP-2, GH effect is thereby inhibiting. At cellular level IGFBP-2 seems to stimulate the proliferation and dissemination of solid tumors via an IGF-independent mechanism. IGFBP-2 is a 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 IGFBPserum levels after remission. During the GH-therapy, e.g. in short stature and in GHabuse (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.

Principles of Testing

The Human IGFBP-2 ELISA Kit is a so-called Sandwich-Assay using two specific and high affinity antibodies. The IGFBP-2 in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-IGFBP-2-Antibody binds in turn to the immobilised IGFBP-2. The second antibody is biotinylated and will be applied in a mixture with a Streptavidin-Peroxidase-Enzyme Conjugate. In the closing substrate reaction the turn of the colour will be catalysed quantitatively depending on the IGFBP-2-level of the samples. It recognizes IGFBP-2 quantitatively and is not influenced by increased IGF-I or IGF-II values. Related molecules such as IGFBP-3 do not show any cross-reactions in the test.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with mouse-anti-hIGFBP-2-antibody. Wells are separately breakable. (8x12) wells
2. **Calibrators (CAL A-E):** lyophilized, (recombinant human IGFBP-2), concentrations are given on vial labels and on quality certificate. 5 x 750 µL
3. **Control 1 (CTR1):** lyophilized, (human serum), concentration is given on quality certificate. 1 x 100 µL

4. **Control 2 (CTR2):** lyophilized, (human serum), concentration is given on quality certificate. 1 x 100 µL
5. **Antibody-HRP-Conjugate (DET):** ready for use, contains rabbit biotinylated anti-hIGFBP-2 antibody + streptavidin horseradish peroxidase conjugate. 1 x 12 mL
6. **Dilution Buffer (DIL):** ready for use. 1 x 50 mL
7. **Washing Buffer (WB):** 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 (STP):** 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 Calibrators A-E 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

The blood sample for serum preparation should be gained according to standardized venipucture procedure. Hemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation. Whole blood should ideally be separated after about 2 hours and processed after 12 hours at the latest and stored at -20 ° C until the measurement. Blood samples may be taken at any time of the day. Haemolytic reactions are to be avoided.

3. Required sample volume: 15 µL

4. Sample stability

In firmly closable sample vials

• Storage at RT max. 24 hours

• Storage at –20°C min. 2 years

Samples are not allowed to have more than 10 freeze/thaw cycles.

5. Interference

Triglyceride, bilirubin and hemoglobin in the sample do not interfere to a concentration of 100 mg/mL, 200 µg/mL or 1 mg/mL, respectively. However, the use of haemolytic, lipemic or icteric samples should be validated by the user.

6. Sample dilution

• Dilution: 1:21 with Dilution Buffer DIL

• Example: 15 µL sample are added to 300 µL Dilution Buffer DIL (dilution factor 21). After mixing this solution, 2 x 100 µL are used in the assay.

• The serum samples must be diluted 1:10 – 1:30 with Dilution Buffer DIL prior to measurement, depending on the expected IGFBP-2 values. In general, a dilution of 1:21 is suitable (and thus the minimum required sample volume for a duplicate determination is 15 µL serum). An extraction step is not required.

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 – E is reconstituted with 750 µL Dilution Buffer DIL. After resuspension, the calibrators are diluted according to a gradient - A (2 ng/mL), B (10 ng/mL), C (20 ng/mL), D (40 ng/mL), E (80 ng/mL), which are prepared for immediate use.
2. The Controls CTR1 and CTR2 are reconstituted with 100 µL Dilution Buffer DIL. After reconstitution dilute the Controls CTR1 and CTR2 with the Dilution Buffer DIL in the same ratio (1:21) 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-E, 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-HRP-Conjugate DET 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-E, 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.

   Manual washing: Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. Decant contents into a biohazard bin, then blot plate on absorbent tissue. Wash the plate by adding 300µL Washing Buffer WB/well, then decant and blot on absorbent tissue. Repeat this step 4 more times for total of 5 washes.

Assay Step

1. Set Calibrator A-E, test samples (1:21 diluted), Controls CTR1 and CTR2 (1:21 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. Aliquot 100ul of Dilution Buffer DIL (Blank), Calibrator A-E, 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 Antibody-HRP-Conjugate DET 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. 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.

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 Calibrator E should be above 1.00. Samples, which yield higher absorbance values than Calibrator E, 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 absorbances of all samples, controls and calibrators.
3. Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis.
4. 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 four parametric logistic (4-PL) curve fit should be used for recalculation of IGFBP-2 concentrations.
5. The IGFBP-2 concentrations of the diluted samples or the diluted control CTR1 & CTR2 in ng/mL is calculated in this way, the IGFBP-2 concentration of the undiluted sample and of CTR1 & CTR2 is calculated by multiplication with the respective dilution factor.

Exemplary calculation of IGFBP-2 concentrations

Sample dilution: 1:21

Measured extinction of your sample 0.37

Measured extinction of the blank 0.06

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 3 rd degree). In this exemplary case the following equation is solved by the program to calculate the IGFBP-2 concentration in the sample:

$$0.31 = -0.0012048 + 0.039581x + 5.1788 \cdot 10^{-0.005} \cdot x^2 - 1.8929x\ 10^{-0.006} \cdot x^3$$

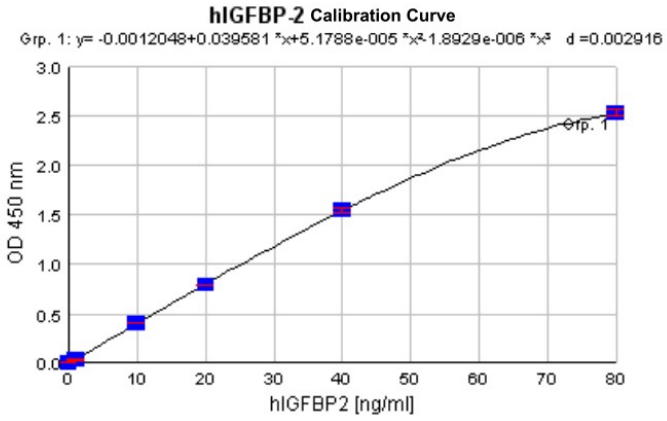
$$x = 7.93$$

If the dilution factor (1:21) is taken into account the IGFBP-2 concentration of the undiluted sample is

$$7.93 \times 21 = 166.55 \text{ ng/mL}$$

Typical Standard Curve

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!



Precision

The inter- and intra assay coefficients of variability are below 10%.

Sensitivity

Sensitivity was assed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the Human IGFBP-2 ELISA Kit is 0.2 ng/mL.

Specificity

The specificity of this ELISA was investigated. Neither 100 ng/mL IGFBP-1 nor 20 µg/mL IGFBP-3 containing samples resulted in a signal significantly different of the blank.

Cross reactions with animal samples

This assay is specific for human IGFBP-2, low degree of cross reactions was found with commercial dog, horse, donkey, cat and goat sera. No cross-reactivity was found with pig, bovine, rabbit, mouse, chicken, rat, guinea pig, sheep sera.

Linearity

The Human IGFBP-2 ELISA Kit can be diluted over a very wide range. The linearity of serum dilutions is shown in table below.

Dilution	Serum 1 (ng/mL)	Serum 2 (ng/mL)
1:10	938	582
1:20	1061	673
1:40	1055	719
1:80	1004	691
1:160	952	668

Recovery

Recombinant IGFBP-2 was added in three different concentrations to human serum. The IGFBP-2 concentration was measured and the mean relative recovery in comparison to buffer was 108%.

Interferences

Interference of bilirubin, triglycerides and hemoglobin was tested by adding different amounts of these substances to human serum containing IGFBP-2. For comparison the same amount of buffer without any substance was also added to the serum. Table below demonstrates that neither bilirubin nor triglycerides exert any influence on the measurement of IGFBP-2 in human serum.

Bilirubin		Triglycerides		Hemoglobin	
[µg/mL]	%	[mg/mL]	%	[mg/mL]	%
25	95.07	12.5	100.79	0.125	99
50	92.80	25	101.01	0.25	105
100	93.83	50	103.65	0.5	100
200	88.15	100	101.34	1	100

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.

   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

Deviation from the reference range can be expected especially in hypothyroidism, after major surgery, in polytrauma, in Diabetes mellitus (due to insulin therapy), in fasting and in malignant diseases. The Human IGFBP-2 ELISA Kit is based on mono- and polyclonal antibodies. Generally the result of any immunological test system can be influenced by heterophilic antibodies, anti-species antibodies or rheumatic factors. The assay design reduces these potential influences to a minimum but they cannot be excluded in any case.