Creative Diagnostics®

Version 07-11/24

User's Manual

Human Insulin-like growth factor II (IGF-II) ELISA Kit

REF **DEIA-NB24-10**

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

Human IGF-II ELISA Kit is suited for measuring IGF-II, human Insulin-like Growth Factor-II, in human serum and plasma for diagnostic and scientific purposes.

General Description

The insulin-like growth factors (IGF)-I and -II play a pivotal role in the regulation of proliferation and differentiation of several tissue types. IGF-I also called Somatomedin C has a molecular weight of 7.469 kDa. Its expression is mainly regulated by Growth Hormone and nutrition. But several hormones and peptide factors are known to influence IGF-II synthesis in different tissues. Bioavailability of the IGFs is regulated by specific binding proteins (IGFBP). Beside the high affinity Insulin-like Growth Factor Binding Proteins 1-6, IGFs are also bound be IGFBP-related Proteins. These binding proteins bind IGF-I and IGF-II with the same affinity or prefer IGF-II. Direct measurement of IGFs in serum samples without pretreatment results in false values because of the extremely slow dissociation of the IGF/IGFBP complexes during the assay incubation only a part of the IGFII in the specimen can bind to the antibodies and be detected.

Therefore, various techniques were applied to physically separate IGF-II from its binding proteins before measurement, including (a) size exclusion chromatography under acidic conditions, (b) solid-phase extraction and (c) acid-ethanol extraction. These techniques, however, are either inconvenient or time-consuming or give incomplete and notreproducible recoveries.

Human IGF-II ELISA Kit is easy, fast and results do not depend on the binding protein concentration of the sample. It is based on the high specificity of the employed antibodies for IGF-II. There is virtually no cross-reactivity with IGF-I. This allows the separation of IGF-II from the binding proteins by acidification and blocking of the free binding proteins with IGF-I. Thus, the endogenous IGF-II is free in solution.

Indication

Scientific studies in the context of neonatal hypertrophy or hypotrophy (IGF-II is a fetal growth factor) and malignancy (IGF-II is an oncogenic growth factor). IGF-II appears to be suitable for differential diagnosis in various malignant diseases. For example, IGF-II can be used to differentiate between adrenocortical tumors and adenomas. In prostate cancer, too, tumor staging and the differentiation between carcinoma and hyperplasia can be improved by measuring IGF-II in serum. Recent results from neurology show that the IGF system is also important in the development of Alzheimer's and Parkinson's disease.

Principles of Testing

The Human IGF-II ELISA Kit is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The first antibody, immobilized on the microtiter plate, and the added second biotinylated antibody are binding the IGF-II in the sample. The Streptavidin-Peroxidase Enzyme Conjugate subsequently binds to the complex. In the closing substrate reaction, the turn of the colour will be high specific catalysed, quantitatively depending on the IGF-II-level of the samples.

IGF-II-IGFBP complex is dissociated by dilution in an acidic buffer. IGFBPs are blocked by IGF-I excess, thus allowing the measurement of free IGF-II. With this method, the IGFBPs are not removed, but their function and therefore their interference in the assay is neutralized. Due to the low cross-reactivity of the IGF-II

antibody with IGF-I, excess IGF-I does not disturb the interaction of the first antibody with IGF-II.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with mouse-anti-hIGF-II-antibody. Wells are separately breakable. (8x12) wells
2. **Calibrators (CAL A-E):** lyophilized, (recombinant human hIGF-II), concentrations are given on vial labels and on quality certificate. 5 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 goat biotinylated anti-hIGF-II antibody. 1 x 6 mL
6. **Enzyme Conjugate (EC):** ready for use, contains HRP (Horseradish-Peroxidase)-labeled Streptavidin. 1 x 12 mL
7. **Sample Buffer (SB):** 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-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

Serum and Heparin/ EDTA/ Citrate Plasma yield comparable values. Possible dilution of the sample by the anticoagulant must be considered.

2. Specimen collection

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided.

3. Required sample volume: 10 µL

4. Sample stability

Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen -20°C or below in tightly closable plastic tubes. Avoid on principal repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although IGF-II levels were found to be unaffected by few cycles in our experiments.

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

Suggestion for dilution protocol:

• Dilution: 1:404 with Sample Buffer SB.

• 1-step dilution: Please pipette 2015 µL Sample Buffer SB in PE/PP-Tubes (applicaton of a multi-stepper is recommended in larger series); subsequently add 5 µL sample (dilution 1:404). Incubate at least for 15 Minutes, max. 2 h, use 50 µL in Assay

• 2-step dilution: Because the pipetting accuracy can rise by the use of 10 µL sample, a 2-step dilution is alternatively possible, for this place 1000 µL Sample Buffer SB in in PE/PP-Tubes, add 10 µL sample (samples are 1:101 diluted), mix from this solution 50 µL with 150 µL Sample Buffer SB (samples are 1:404 diluted). Incubate at least for 15 Minutes, max. 2 h, use 50 µL in 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 – E is reconstituted with 500 µL Sample Buffer SB. After resuspension, the calibrators are diluted according to a gradient - A (0.45 ng/mL), B (1.5 ng/mL), C (3 ng/mL), D (5.63 ng/mL), E (9 ng/mL), which are prepared for immediate use.
2. The Controls CTR1 and CTR2 are reconstituted with 250 µL Sample Buffer SB. After reconstitution dilute the Controls CTR1 and CTR2 with the Sample Buffer SB in the same ratio (1:404) as the sample. Mix immediately, incubate at least for 15 minutes, max. 2 h.

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, Blank, Calibrators A-E, Controls 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 (Blank, 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. 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-E, test samples (1:404 diluted), Controls CTR1 and CTR2 (1:404 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 Sample Buffer SB (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 2 hours.

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 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 absorbance of all other 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. Recommendation: Calculation of the calibrator 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 IGF-I concentration in ng/mL of the samples can be calculated by multiplication with the respective dilution factor.

Exemplary calculation of IGF-II concentrations

Sample dilution: 1:404

Measured extinction of your sample 0.449

Measured extinction of the blank 0.0329

Your measurement program will calculate the IGF-II concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit.

In this exemplary case the following equation is solved by the program to calculate the IGF-II concentration in the sample:

$0.4161 = -0.0115x^2 + 0.3136x - 0.0433$

$x = 1.49$

If the dilution factor (1:404) is taken into account the IGF-II concentration of the undiluted sample is

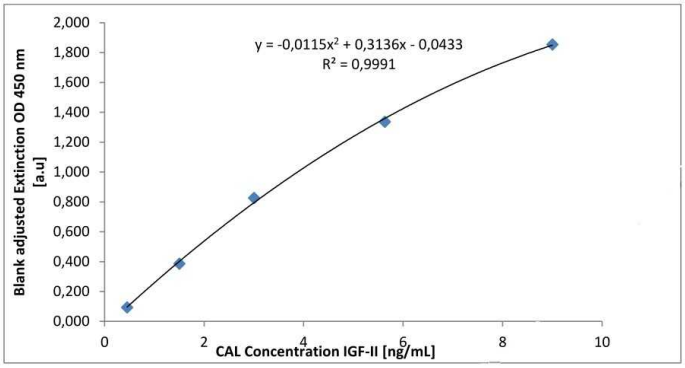
1.49 ng/mL × 404 = 601.96 ng/mL

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
ng/mL	0	0.45	1.5	3	5.63	9
$OD_{(450\text{-}620\ nm)}$	0.0329	0.127	0.4195	0.8595	1.3695	1.855

The exemplary shown standard 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 variation coefficients are in mean <10%.

Intra-Assay Variation

	Determinations [n]	Mean value [ng/mL]	SD	CV [%]
Sample 1	18	1019	86.4	8.49
Sample 2	18	692	55.4	8.00
Sample 3	18	570	41.2	7.28

Inter-Assay Variation

	Determinations [n]	Mean value [ng/mL]	SD	CV [%]
Sample 1	16	666	20	3.07
Sample 2	16	875	58	6.61
Sample 3	20	621	26	4.17
Sample 4	19	670	41	6.13
Sample 5	20	795	42	5.29

Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the 2-fold standard deviation of the blank. The mean analytical sensitivity of the Human IGF-II ELISA Kit is 0.06 ng/mL (Range 0.01 - 0.223 ng/mL, n = 7)

Specificity

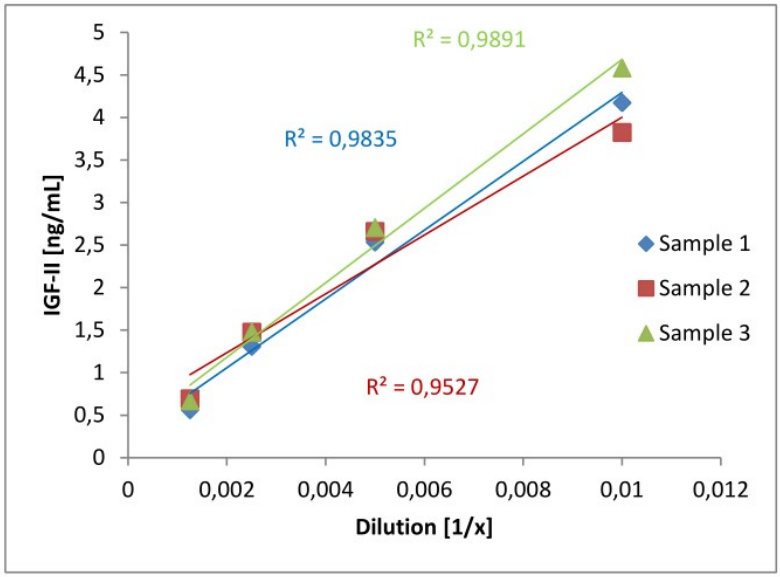
IGF-I, a homologous molecule to IGF-II, was diluted in three different concentrations (250, 750, 1250 ng/mL) and tested with the IGF-II ELISA. None of the samples gave a significant signal.

Cross reactions with animal samples

It has been shown that the test can be used as a heterologous assay for IGF-II measurement in serum samples from bovine and pigs. Species-specific calibration must be carried out by the user.

Linearity

Three serum samples were measured in different dilutions. Standard assay dilution is 1:404. Here sample dilutions of 1:100 – 1:800 were used. These dilutions cover a concentration range of 4.5 to 0.6 ng/mL.



Interferences

Interference of haemoglobin, bilirubin and triglycerides was tested by adding different amounts of these substances to human serum containing IGF-II. For comparison the same amount of buffer without any substance was also added to the serum. Table below demonstrates that there is no significant influence of these substances on the measurement of IGF-II in human serum.

	Triglyceride (100 mg/mL)	Bilirubin (100 µg/mL)	Hemoglobin (1 mg/mL)
Sample 1	97	103	102
Sample 2	95	95	91
Sample 3	90	111	119

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

The Human IGF-II ELISA Kit is based on specific antibodies. Generally, this technique is sensible to heterophilic antibodies in the sample. The influence of heterophilic antibodies is reduced by assay design, but cannot be excluded completely.