Creative Diagnostics®

Version 07-11/24

User's Manual

Mouse/Rat Insulin-like growth factor I (IGF-I) ELISA Kit

REF **DEIA-NB24-09**

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

The Mouse/Rat IGF-I ELISA Kit is intended to be used for the measurement of mouse and rat IGF-I in serum and plasma samples.

General Description

Beside different cell culture models and studies with human material, mice and rats are suitable model organisms for basic research and pre-clinical studies. Thus, we developed this test system as a tool for IGF-I measurements in mice and rat for usage in research and pre-clinical studies. Even if the comparability of mice and humans is limited, we offer some background information on the human IGF-I system in the following section.

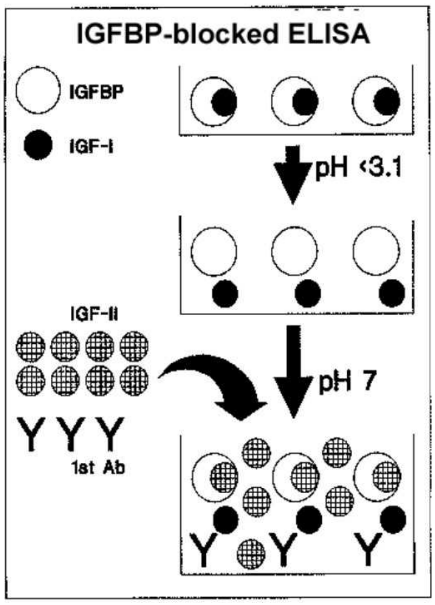
Insulin-like growth factors (IGF) I and II play a pivotal role in regulating the proliferation and differentiation of many cell types. IGF-I is identical with Somatomedin C (Sm-C) and has a molecular weight of 7649 daltons. Its major regulators are growth hormone (GH) and nutrition. In contrast to many other peptide hormones, IGFs are avidly bound to specific binding proteins (IGFBP). The seven IGFBPs which are known at present either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II. A major problem of IGF-I measurement results from the interference of IGFBPs in the assay. Direct determinations in untreated serum samples give false values because of the extremely slow dissociation of the IGF-I/IGFBP-3 complexes during the assay incubation.

To avoid these difficulties, an uncomplicated assay was developed, in which special sample preparation, except acidification or dilution in a specially designed buffer system, is not required before measurement.

Principles of Testing

The Mouse/Rat IGF-I ELISA Kit is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The IGF-I in the sample binds to the immobilized first antibody on the microtiter plate, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-IGF-I-Antibody binds in turn to the immobilized IGF-I. In the closing substrate reaction, the turn of the colour will be high specific catalysed, quantitatively depending on the IGF-I-level of the samples.

In order to dissociate IGF-I from the IGFBPs, the samples must be diluted in an acidic buffer (Figure below). The diluted samples are then pipetted into the wells, by this the pH-value will be neutralized. After neutralization of the samples, the excess IGF-II occupies the IGF-binding sites of the binding proteins, thus allowing the measurement of resulting free IGF-I. With this method, the IGFBPs are not removed, but their function and therefore their interference in the assay is neutralized. Due to the extremely low cross-reactivity of the IGF-I antibody with IGF-II, the excess of IGF-II does not disturb the interaction with IGF-I. The test runs like a conventional ELISA using a Streptavidin-Peroxidase-Enzyme Conjugate.



Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with hamster anti-Mouse/Rat IGF-I antibody. Wells are separately breakable. (8x12) wells
2. **Standards (A-E):** lyophilized, (recombinant IGF-I), concentrations are given on vial labels and on quality certificate. 5 x 1 mL
3. **Control Serum 1 (KS1):** lyophilized, (Mouse/Rat serum), concentration is given on quality certificate in ng/mL. 1 x 500 µL
4. **Control Serum 2 (KS2):** lyophilized, (Mouse/Rat serum), concentration is given on quality certificate in ng/mL. 1 x 500 µL
5. **Antibody Conjugate (AK):** ready for use, contains goat biotinylated anti-mouse/rat IGF-I antibody. 1 x 7 mL
6. **Enzyme Conjugate (EK):** ready for use, contains HRP (Horseradish-Peroxidase)-labeled Streptavidin. 1 x 12 mL
7. **Sample Buffer (PP):** ready for use. 1 x 125 mL
8. **Washing Buffer (WP):** 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 (SL):** 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 standards A-E and Control Sera KS1 and KS2 must be stored at –20°C (max. 2 Months).

Attention: Standards should be thawed only once – where required please store aliquoted in adequate volumes. For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Avoid repeated thawing and freezing. 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 samples as well as Heparin-, EDTA- and Citrat-Plasma samples are suited. Possible dilution of the sample by the anticoagulant must be considered.

*Influence of Heparin (30IE/mL), EDTA (6,8mM) and NaCitrat (0,015M) on the measurement of IGF-I has been investigated in recovery experiments. Buffer solution was enriched with recombinant IGF-I and the above-mentioned substances. No significant influence on the recovery of IGF-I was detected, on average the recovery of recombinant material in comparison to enriched PBS was 108%.

*Cell culture medium is suitable as sample matrix after predilution of 1:2 with Sample Buffer PP.

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

In firmly closable sample vials

• Storage at 20-25°C: max. 2 days

• Storage at -20° C: max. 2 years

• Freeze-thaw cycles max. 2

The storage of samples over a period of 2 years at -20°C, showed no influence on the reading. It is recommended to keep sample refrigerated or frozen as soon as possible after separation of coagulated and corpuscular blood components and to avoid more than 2 freeze-thaw cycles.

5. Sample dilution

• Dilution: 1:100 with Sample Buffer PP

• Pipette 990 µL Sample Buffer PP in PE-/PP-Tube (application of a multi-stepper is recommended in larger series);

add 10 µL sample (dilution 1:100). After mixing use 50 µL of this dilution in the assay.

• Attention: serum and plasma samples must be diluted at least 1:10 in Sample Buffer PP in order to achieve sufficient acidification of the samples.

• Depending on the expected IGF-I values the samples can be diluted higher or lower in Sample Buffer PP.

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 – E is reconstituted with 1mL Sample Buffer PP. After resuspension, the standard is diluted according to a gradient - A (0.5 ng/mL), B (2.5 ng/mL), C (6 ng/mL), D (12 ng/mL), E (18 ng/mL), which are prepared for immediate use.
2. The Control Sera KS1 and KS2 are reconstituted with 500 µL Sample Buffer PP. After reconstitution dilute the Controls KS1 and KS2 with the Sample Buffer PP 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-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 AK and the 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-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 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.

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 300 µL Washing Buffer WP/well, then decant and blot on absorbent tissue. Repeat this step 4 more times for total of 5 washes.

Assay Step

1. Set Standard A-E, test samples (1:100 diluted), Control Serum KS1 and KS2 (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. Add 50ul Antibody Conjugate AK in all wells used. Aliquot 50ul of Sample Buffer PP (Blank), Standard A-E, Control Serum KS1/KS2 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 Enzyme Conjugate EK into above wells (standard, control serum and test samples). 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 WP. 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 within 30 minutes.
8. Stop: Add 100ul Stopping Solution SL into each well.
9. Measure the absorbance within 30 min at 450 nm, with ≥ 590 nm as reference wavelength.

Quality Control

For the evaluation of the assay it is required that the absorbance values of the should be below 0.25, and the absorbance of standard E should be above 1.00. Samples, which yield higher absorbance values than Standard 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 ssamples and standards.
3. Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4. 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 four parametric logistic (4-PL) curve fit should be used for recalculation of IGF-I concentrations. .
5. The IGF-I concentration of the diluted sample or the diluted control sera KS1&2 in ng/ml (or µg/ml according the chosen unit for the standards) is calculated in this way, the IGF-I concentration of the undiluted sample and of KS1 & KS2 is calculated by multiplication with the respective dilution factor.

Exemplary calculation of IGF-I concentrations

Sample dilution: 1:100

Measured extinction of your sample 1.3525

Measured extinction of the blank 0.07

Your measurement program will calculate the IGF-I 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-I concentration in the sample:

$1.283 = 0.0004x^3 – 0.0184x^2 + 0.3029x$

$6.107 = x$

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

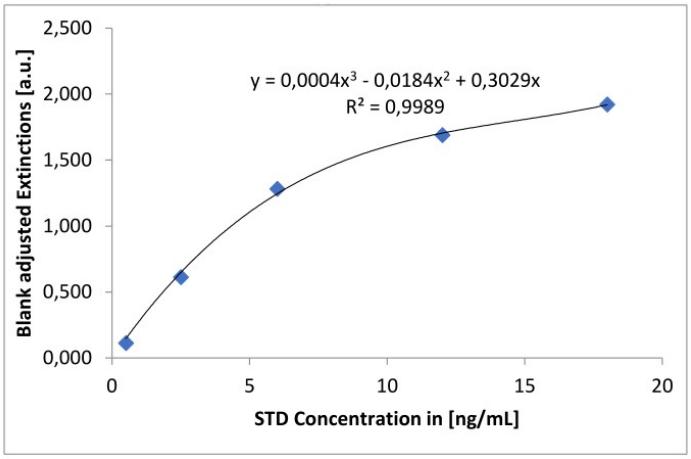
$6.107 \times 100 = 610.7$ ng/mL

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	0.5	2.5	6	12	18
$OD_{(450\text{-}620\ nm)}$	0.07	0.114	0.614	1.283	1.690	1.923

The exemplary shown standard curve in Figure 3 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

Several samples were measured 16 in the same assay. On average, the variation coefficient was <10%.

	Determinations [n]	Mean value [µg/L]	SD	CV [%]
Sample 1	16	246	13.09	5.32
Sample 2	16	684	52.27	7.64
Sample 3	16	679	93.61	13.79

Inter-Assay-Variability

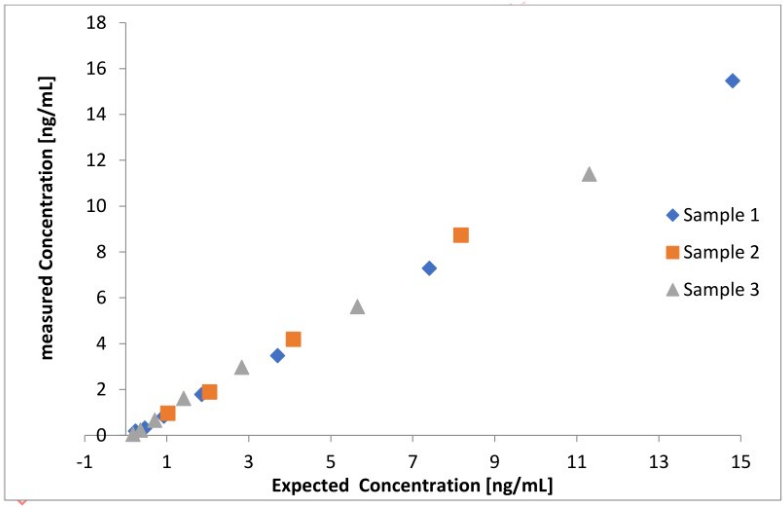
Serum samples were measured in independent assays. On average, the coefficient of variation <10%.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
Determinations [n]	24	26	23	23	26	26	26	26	26	26
Mean value [µg/L]	291	695	773	256	151	444	127	686	581	178
SD	10	51	76	15	21	37	12	59	50	12
CV [%]	7	7	10	6	14	8	69	9	9	7

Sensitivity

The sensitivity was determined by measuring the blank value and calculating the theoretical concentration of the blank value +2 SA. The analytical sensitivity of the Mouse/Rat IGF-I ELISA Kit is as an average 0.315 ng/mL (range 0.262-0.405 ng/mL).

Linearity



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 Mouse or Rat serum: Control Serum KS1 and KS2.

Tel: 1-631-624-4882 (USA)
Tel: 44-161-818-6441 (Europe)
Fax: 1-631-938-8221
Email: info@creative-diagnostics.com