



Mouse/Rat IGFBP-2 ELISA

**For the quantitative determination of IGFBP-2
in serum of mice and rats.**

Please be aware of updates to technical notes and storage conditions.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	22-BP2MS-E01
Size:	96 Wells
Version:	V11 25.03.2015 – ALPCO August 31, 2015

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	INTERNATIONAL ASSAY DESCRIPTION	ERROR! BOOKMARK NOT DEFINED.

22-BP2MS-E01	96 Determinations
Type of the test	Sandwich ELISA
Duration (incubation period)	3 hours
Antibody Conjugate	100-fold concentrate
Enzyme Conjugate	100-fold concentrate
Buffer and Substrate	Ready for use
Standards	7 single standards: 31.25 - 2000 pg/mL, lyophilized, native mouse IGFBP-2
Assay Range	0.01 –200 ng/mL
Control	1 Control Serum, lyophilized
Sample	Serum from rat and mouse
Required sample volume	10 µL
Sample dilution	1:100
Analytical sensitivity	0.01 ng/mL
Average Intra- / Inter-Assay Variance	< 10%

1 INTENDED USE

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

2 INTRODUCTION

Insulin-like growth factors (IGFs) regulate the proliferation, differentiation, apoptosis, cell adhesion and metabolism in various tissues and cell types.

IGFBP-2 is an unglycosylated polypeptide 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 (5-10). 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 (17).

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 (18). Effects of IGFBP-2 on the organism can be compensated through the modified expression of other IGF-Binding proteins.

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 (19). Furthermore IGFBP-2 interacts with tumor cells via its RGD-amino acid sequence and seems to stimulate cell invasion of glioma cells (20).

3 ASSAY PRINCIPLE

The Mouse/Rat IFGBP-2 ELISA, 22-BP2MS-E01, 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.

4 WARNINGS AND PRECAUTIONS

For Research Use only. For Professional use only.

The Mouse/Rat IGFBP-2 ELISA kit is suitable only for Research Use Only and not for internal use in humans and animals. Follow the test protocol strictly. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. The manufacturer will not be held responsible for any loss or damage (except as required by statute) caused or arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore all components and samples should be treated as potentially infectious.

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.

Reagents AK, EK, VP, WP

Contain as preservative **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local, regional, national, and international regulations.

Substrate Solution (S)

The TMB-Substrate (S) contains 3,3',5,5' Tetramethylbencidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

Stopping Solution (SL)

The Stopping solution contains 0.2 M acid Sulphur acid (H₂SO₄)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves, protective clothing, eye protection, and face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.

P338 Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310 IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

5 SAMPLES

5.1 **Sample type:** Mouse and Rat Serum and Plasma

5.2 **Specimen collection:** Hemolytic reactions have to be avoided.

5.3 **Requested sample volume:** 10 µl serum.

5.4 Sample stability

- In firmly closable sample vials
- 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.

5.5 Interference

Hemoglobin in the sample does not interfere to a concentration of **5 mg/mL**.

However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

5.6 Sample dilution


Samples must be diluted prior to measurement. An extraction step is not required.

- **Dilution:** 1:100 with Dilution Buffer **VP**
- **Example:** Pipette **990 µL Dilution Buffer VP** in PE-/PP-Tubes add **10 µL Serum** (dilution factor 100).
- 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**
- After Mixing use 100 µL of this dilution in the assay.
- Serum samples should be diluted prior to measurement 1:20 – 1:500-fold with Dilution Buffer **VP**, depending on the expected values (see chapter Expected Values).

6 MATERIALS

6.1 Materials provided

The reagents listed below are sufficient for 96 wells including the standard curve.

MTP	Microtiter plate , ready for use, coated with rabbit-anti-IGFBP-2 antibody. Wells are separately breakable.	(8x12) wells
A-G	Standards , lyophilized (native mouse IGFBP-2), concentrations are given on the vial labels and on the QC-certificate.	7 x 1 mL
KS	Control Serum , lyophilized, (mouse serum), the concentration is given on the QC-Certificate .	1x 250 µL
AK	Antibody Conjugate 100-fold concentrated, goat-anti-mIGFBP-2-Antibody biotinylated	1 x 120 µL
EK	Enzyme Conjugate (POD) 100-fold concentrated, Streptavidin Peroxidase-Conjugate	1 x 120 µL
VP	Dilution Buffer , ready for use. Please shake before use!	1 x 120 mL
WP	Washing Buffer , 20-fold concentrated solution	1 x 50 mL
S	Substrate , ready for use, horseradish-peroxidase-(HRP) substrate	1 x 12 mL
SL	Stopping Solution , ready for use, 0.2 M sulphuric acid.	1 x 12 mL
-	Sealing Tape , for covering the microtiter plate	2 x
	Instructions for use	1 x
-	Quality Control Certificate (QC-certificate)	1 x

6.2 Materials required, but not provided

- Distilled or deionized water for dilution of the Washing Buffer **WP** 950 mL.
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥ 590 nm
- Timer

7 TECHNICAL NOTES

Storage Conditions

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.

Storage Life

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

Preparation of reagents

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.

Reconstitution

The Standards **A – G** and Control **KS** are reconstituted with the Dilution Buffer **VP**. 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.

Dilution

After reconstitution dilute the Control Serum **KS** with the Dilution Buffer **VP** in the same ratio (1:100) as the sample. The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20-fold concentrate with Distilled water.

Assay Procedure Notes

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

Incubation

Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate Solution **S**, stabilized H₂O₂-Tetramethylbenzidine, is photosensitive—store and incubation in the dark.

Shaking

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must 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.

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.

When using an **automatic microtiter** plate washer, the respective instructions for 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 is an adequate alternative option. 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.

8 PROCEDURE

Preparations of Reagents

Before assay procedure, bring all reagents to room temperature **20-25°C**.

1. Standards A-G

Reconstitute each standard A-G with **1 mL** Dilution Buffer **VP**.

2. Control Serum KS

Reconstitute with **250µL** of Dilution Buffer **VP**. Dilute reconstituted Control Serum **KS** 1:100 with Dilution Buffer **VP**.

3. Antibody Conjugate AK

Dilute Antibody Conjugate **AK** **1:100** with Dilution Buffer **VP**.

4. Enzyme Conjugate EK

Dilute Enzyme Conjugate **EK** **1:100** with Dilution Buffer **VP**.

5. Washing Buffer WP

Dilute Washing Buffer **1:20** with Distilled Water.

6. Sample Dilution

Dilute Samples with Dilution Buffer **VP** **1:100**

Assay Procedure

- 1) Add **100 µl Dilution Buffer VP** in wells A1&2 (blank) and
- 2) pipette in positions B1&2 **100 µl Standard A (31.25 pg/mL)**, pipette in positions C1&2 **100 µl Standard B (62.5 pg/mL)**, pipette in positions D1&2 **100 µl Standard C (125 pg/mL)**, pipette in positions E1&2 **100 µl Standard D (250 pg/mL)**, pipette in positions F1&2 **100 µl Standard E 500 pg/mL)**, pipette in positions G1&2 **100 µl Standard F (1000 pg/mL)**, pipette in positions H1&2 **100 µl Standard G (2000 pg/mL)**. pipette in positions A3&4 **100 µl Control Serum (diluted 1:100)** pipette **100 µl** of the **diluted sample** in the rest of the wells, according to requirements.
- 3) Cover the wells with sealing tape and incubate the plate for **1 hour (60 minutes)** at **20-25°C** (shake at 350 rpm).
- 4) After incubation, aspirate the contents of the wells and wash the wells **5 times** with **300 µl** of **Washing Buffer WP** well respectively.
- 5) Following the last washing step, pipette **100 µl** of the of the **(1:100)** diluted **Antibody Conjugate AK** in each well, cover with sealing tape, and incubate **1 hour (60 minutes)** at **room temperature** (shake at 350 rpm).
- 6) After incubation, aspirate the contents of the wells and wash the wells **5 times** with 300 µl of **Washing Buffer WP** as described in step 4.
- 7) Following the last washing step pipette **100 µl** of the **(1:100)** diluted **Enzyme Conjugate EK** in each well. Cover the wells with sealing tape. Incubate **0.5 hour (30 minutes)** at **20-25°C** (shake at 350 rpm).
- 8) After incubation wash the wells **5 times** with **300 µl** of **Washing Buffer WP** as described in step 4.
- 9) Pipette **100 µl** of the **TMB-Substrate Solution S** in each well.
- 10) Incubate the plate for **0.5 hour (30 minutes)** in the dark at **20-25°C**.
- 11) Stop the reaction by adding **100 µl** of **Stopping Solution SL** to all wells.
- 12) Measure the absorbance within **0.5 hour (30 minutes)** at **450 nm (reference filter: ≥ 590 nm)**.

Procedure Summarized

Preparation of reagents:		Reconstitution:	Dilution:
Before assay procedure bring all reagents to room temperature 20-25°C .			
A-G	Standards	in 1 mL Dilution Buffer VP	-
KS	Control Serum	in 250 µL Dilution Buffer VP	1:100 with Dilution Buffer VP
AK	Antibody Conjugate	-	1:100 with Dilution Buffer VP
EK	Enzyme Conjugate	-	1:100 with Dilution Buffer VP
WP	Washing Buffer	-	1:20 with distilled water .
Sample dilution: with Dilution Buffer VP 1:100			
Assay Procedure in Double Determination:			
Pipette	Reagent		Position
100 µL	Dilution Buffer VP (Blank)		A1/A2
100 µL	Standard A (31.25 pg/mL)		B1/B2
100 µL	Standard B (62.5 pg/mL)		C1/C2
100 µL	Standard C (125 pg/mL)		D1/D2
100 µL	Standard D (250 pg/mL)		E1/E2
100 µL	Standard E (500 pg/mL)		F1/F2
100 µL	Standard F (1000 pg/mL)		G1/G2
100 µL	Standard G (2000 pg/mL)		H1/H2
100 µL	Control Serum	(1:100 diluted)	A3/A4
100 µL	Sample	(1:100 diluted)	Pipette in the rest of the wells according the requirements
Cover the wells with the sealing tape.			
Sample Incubation: 1 hour at 20-25°C, 350 rpm			
5x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well.		In each well
100 µL	Antibody Conjugate AK (1:100 diluted)		In each well
Cover the wells with the sealing tape.			
Incubation: 1 hour at 20-25°C, 350 rpm			
5x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well.		In each well
100 µL	Enzyme Conjugate EK (1:100 diluted)		In each well
Cover the wells with the sealing tape.			
Incubation: 0.5 hour (30min) at 20-25°C, 350 rpm			
5x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well.		In each well
100 µL	Substrate Solution S		In each well
Substrate S Incubation: 0.5 hour (30min) in the Dark at 20-25°C			
100 µL	Stopping Solution SL		In each well
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.			

9 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.

9.1 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.

10 EVALUATION OF RESULTS

10.1 Establishing of the standard curve

The standards provided contain the following concentrations of native mIGFBP-2:

Standard	A	B	C	D	E	F	G
pg/mL	31.25	62.5	125	250	500	1000	2000
ng/mL	0.03125	0.0625	0.125	0.25	0.5	1	2

- 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**.

10.2 Example of a 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 (450-620 nm)	0.112	0.042	0.083	0.159	0.325	0.632	1.237	2.27

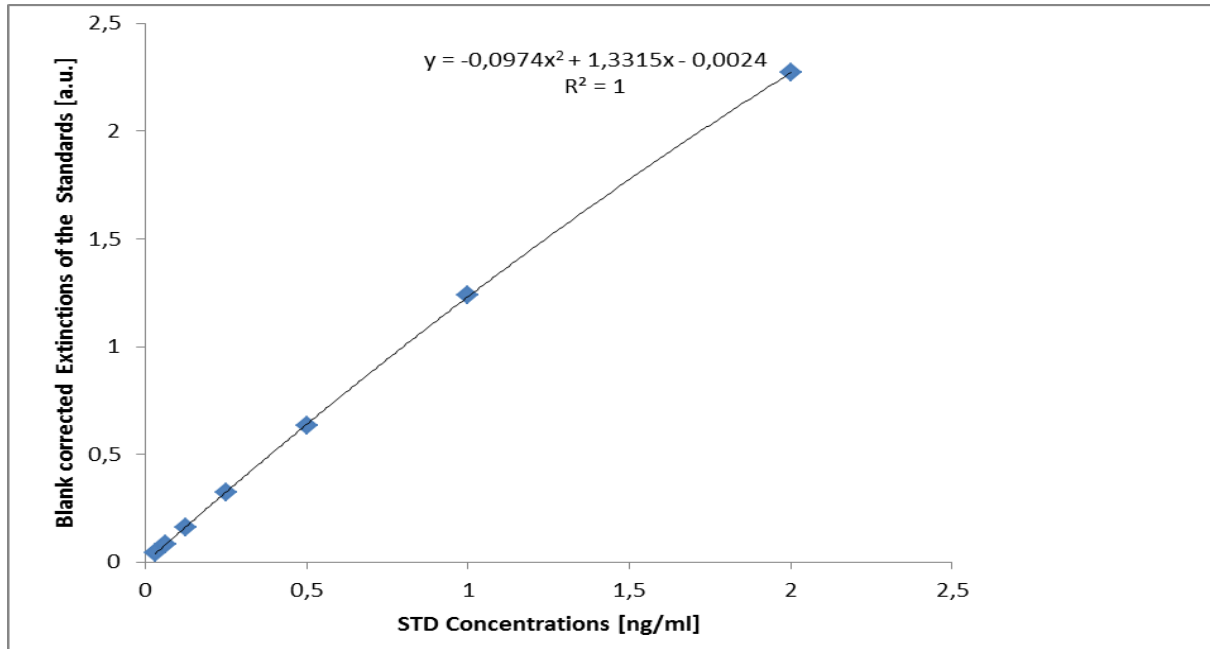


Figure 1: Exemplary standard curve

The exemplary shown standard curve in Figure 1 **cannot** be used for calculation of the test results. Establish a standard curve for each test conducted!

10.3 Exemplary calculation of IGFBP-2 concentrations

Sample dilution: 1:100

Measured extinction of the sample 0.961

Measured extinction of the blank 0.112

The measurement program will calculate the IGFBP-2 concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. One has 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:

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

$$0.673 = x$$

If the dilution factor (**1:100**) is taken into account the IGFBP-2 concentration of the undiluted sample is 0.673 ng/mL \square 100 = 67.3 ng/mL

11 EXPECTED VALUES

Several commercially available mouse and rat sera have been tested for their IGFBP-2 concentrations, following results were obtained:

Table 1: Expected values IGFBP-2 serum concentrations from 5 mouse- and 4 rat sera

	n	Median	min.	max.
Mouse Sera	5	81.0 ng/ml	38.1 ng/mL	105.7 ng/mL
Rat Sera	4	24.2 ng/ml	10.7 ng/mL	59.7 ng/mL

Significant variations of serum values depending on the individual animal or the respective strain or mutant are likely, prior verification is recommended.

12 PERFORMANCE CHARACTERISTICS

12.1 Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank +2SD. The analytical sensitivity of the E08 is 0.01 ng/ml.

12.2 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.

12.3 Reproducibility and Precision

Intra-assay-Variation

Four serum samples were measured in 16 independent assays. On average the coefficient of variation was 5.1% (SD 1.3). Exemplary results are shown in table 2.

Table 2: Intra-Assay Variation

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

Inter-assay-Variation

Four serum samples were measured in 16 independent assays. On average the coefficient of variation was 5.1% (SD 1.3). Exemplary results are shown in table 3.

Table 3: Inter-Assay Variation

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

12.4 Linearity

Three different serum samples were diluted and the recalculated m/rIGFBP-2 concentration measured in each dilution is compared to the theoretically expected value (Figure 2). Samples were diluted from 1:5 up to 1:320.

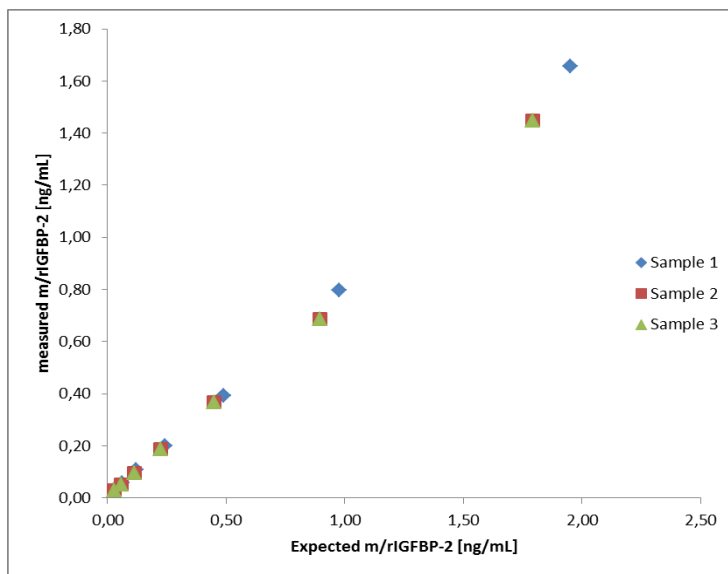


Figure 2: Linearity of the sample dilution: Shown are the measured concentrations in different dilutions of a) mouse serum and b) rat serum samples.

12.5 Species Cross-Reactivity

Species Cross-Reactivity

Several commercially available animal sera have been used as samples (1:10/1:100) in this assay. A signal was detected in the serum of the following species:

Horse, guinea pig, dog, cat, donkey, goat

No signal was detected in:

Pig, bovine, rabbit, chicken, sheep samples

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