BIOSOURCE

Mouse
IGFBP-2 ELISA

Enzyme Immunoassay for Quantitative Determination of

Mouse-Insulin-like Growth Factor Binding Protein-2

Product Code: KAPME08
(96 Determinations)

For Research Use Only!

TECHNICAL FEATURES

- Highly specific and sensitive assay for quantitative detection of IGFBP-2 in mouse serum
- Recombinant mouse IGFBP-2 as standard
- Uses antibodies against complete mouse IGFBP-2
- No sample extraction is required
- Detection limit: 0.04 ng/ml

INTRODUCTION

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 foetal tissue (11-13). The biological actions of IGF over the IGF-Type-I receptor are modulated variably through the IGF binding proteins (IGFBP-1 to-6) (14). 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 (15,16).

PHYSIOLOGICAL MEANING

IGFBP-2 is an 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 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 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.

Especially in tumor biology the mouse system enables 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).

INTENDED USE

This IGFBP-2 Enzyme Immunoassay-Kit is suited for quantitative determination of IGFBP-2 in mouse serum for scientific purposes. Several rat sera (3 different ones) were used in this assay, but no IGFBP-2 could be proved.

METHODOLOGY

Assay Characteristics and Validation

The ELISA for IGFBP-2 utilizes a specific high affinity polyclonal antibody and a specific monoclonal antibody for this protein. The ELISA recognizes quantitatively mouse-IGFBP-2 and is unaffected by an excess of IGF-I or IGF-II levels. Related molecules such as IGFBP-3 show no cross-reactions in the assay.

The standards are prepared of recombinant mouse-IGFBP-2 in the range of 0.125 to 8 ng/ml.

The theoretical sensitivity of the assay is 0.04 ng/ml (2 x SD of zero standard). Intra-assay and inter-assay variation coefficients were found both < 10%. Exemplary determinations are shown in the tables 1 and 2.

Table 1 : Inter-assay-Variation

Sample 1 (ng/ml)	538	523	538
Sample 2 (ng/ml)	958	949	937

Table 2: Intra-assay-Variation

Sample 1 ng/ml	130	161	157	161	138	160	126	162	153
Sample 2 ng/ml	139	115	133	138	126	111	113	123	115

Table 3: Linearity of the sample dilution:

Dilution	**Serum 1 (ng/ml)**	**Serum 2 (ng/ml)**	**Serum 3 (ng/ml)**
1:100	181	443	403
1:200	197	448	426
1:400	207	473	384

Calibration

The assay has been calibrated against the recombinant Mouse-IGFBP-2 of R&D Systems Inc. (Minneapolis, USA; www.rndsystems.com).

Sample Preparation and Storage

Whole blood should be processed within two hours. Once separated the samples should be stored frozen until measurement. IGFBP-2 levels are influenced by improper handling or storage and do not remain stable over several days at elevated temperatures. Store undiluted samples frozen in a tightly closed plastic vial. **Repeated freezing and thawing of serum/plasma should be avoided,** it seems to have a measurable effect on IGFBP-2 levels.

The high sensitivity of the assay allows measurement of IGFBP-2 in small sample volumes, which is limited by pipetting accuracy rather than the amount of IGFBP-2.

Serum samples should be diluted prior to measurement 1:100-500-fold with **Dilution Buffer** depending on the expected values. In general a dilution of 1:100 is appropriate (the recommended minimal essential sample volume is: 10 µl serum). Sample extraction is not required.

Suggestion for dilution protocol (double determination):

Mix 2.5 µl serum manually or with the aid of a dilutor with 247.5 µl **Dilution Buffer** (1:100). Use 2 x 100 µl of this dilution in the assay.

MATERIALS

Materials Provided

1) **Microtiter Plate**, ready for use: Microtiter plate with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with anti-IGFBP-2 antibody and packed in a laminate bag.

2) **Standards 1-7,** lyophilized: Contain recombinant mouse IGFBP-2: Standard values are between 0,125 - 8 ng/ml IGFBP-2 and have to be reconstituted with **1 ml Dilution Buffer** each.

3) **Control 1,** lyophilized: Contains mouse serum and has to be reconstituted with **100 µl Dilution Buffer**. The exact concentration is given on the vial label.

4) **Dilution Buffer**, 120 ml, ready for use.

5) **Biotin Conjugate**, 120 µl, 100fold concentrated: Contains biotinylated anti-IGFBP-2 antibody and has to be diluted immediately before use **1:100** with **Dilution Buffer**.

6) **HRP Conjugate,** 120 µl, 100fold concentrated: Contains HRP-labelled Streptavidin and has to be diluted immediately before use **1:100** with **Dilution Buffer**.

7) **Washing Buffer**, 50 ml, 20fold concentrated: Washing Buffer has to be diluted **1:20** with distilled water before use.

8) **Chromogenic Substrate,** 12 ml, ready for use.

9) **Stopping solution**, 0.4 N sulphuric acid, 12 ml, ready for use. *Caution, acid!*

10) **Sealing tape** for covering of the Microtiter plate, 2 x

Materials not Provided

- Distilled or demineralized water for dilution of the Washing Buffer
- Micropipettes and multichannel pipettes with disposable plastic tips
- Vortex-mixer
- Device to aspirate the standards and the samples from the wells
- Plate washer and plate shaker (recommended)
- Microplate reader ("ELISA-Reader") with filter for 450/620 nm wavelengths
- Foil welding device for laminate bags (recommended)

TECHNICAL RECOMMENDATIONS

In conducting the assay, follow strictly the test protocol.

Reagents with different lot numbers should not be mixed.

The microtiter plate and all reagents are stable until the expiry date if stored in the dark at 2-8°C (s. label).

The **Dilution Buffer** should be used for the **reconstitution** of the lyophilized components **(Standards 1 - 7** and **Control 1)**. 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.
The **shelf life** of the components **after opening** is not affected, if used appropriately. Store the unused seal stripes of the microtiter plate together with the desiccant at 2-8°C.
Reconstituted components (**Standards 1 – 7** and **Control 1**) should be stored at 2-8°C for up to 1 week. If longer storage time is needed, store the components frozen at -20°C or below. Freezing extends the expiry at least 2 months.

Avoid repeated freeze-thaw cycles. In case you plan to perform multiple independent mIGFBP-2 determinations over a longer period with one kit, you should aliquot the components prior to freezing into suitable smaller volumes. This is strongly recommended.

The 1:20 diluted **Washing Buffer** is stable only limited. Please dilute only according to requirements.
This applies to the 1:100 diluted **biotin conjugate** and **HRP Conjugate** solutions too.

Before use, all kit components should be brought **to room temperature**. Room temperature incubation means: incubation at 20 - 25°C. Precipitates in buffers should incase be dissolved before use thorough mixing and warming.

The **Chromogenic substrate**, stabilised H_2O_2-Tetramethylbenzidine, is photosensitive – store and incubate in the dark.

When performing the assay, the **Standards 1-7**, **Control 1** and the samples should be pipette as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times, the 1:100 diluted **biotin conjugate** and the **HRP Conjugate** solutions as well as the succeeding **Chromogenic substrate** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution** should be added to the plate in the same order as the **Chromogenic substrate**.

PRECAUTIONS

The kit should not be used beyond the expiration date on the kit label.

All reagents are for in vitro use only!

In conducting the assay, follow strictly the test protocol. The acquisition, possession and use of the kit are subjects to the regulations of the national regulatory authorities.

Reagents with different lot numbers should not be mixed.

The Stop Solution provided is an acid solution. Avoid direct contact. Wear eye, hand, face and clothing protection when using this material.

The handling of potentially infectious material must comply with the following guidelines:

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

Reagents contain as preservative Thimerosal, however, highly diluted (0.01%). Thimerosal is very toxic when swallowed and it involves a certain danger of cumulative effects (R-Phrases 26/27/28-33-50/53 and S 13-28.1-36-45-60-61).

First aid procedures:

Skin contact: Wash affected area thoroughly with water. Discard 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: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

ASSAY PROCEDURE

All determinations (**Standards, Control 1** and **samples**) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

1) add **100 µl Dilution Buffer** in wells A1/2 (blank) and

2) pipette in positions B1/2 **100 µl Standard 1,**
   pipette in positions C1/2 **100 µl Standard 2,**
   pipette in positions D1/2 **100 µl Standard 3,**
   pipette in positions E1/2 **100 µl Standard 4,**
   pipette in positions F1/2 **100 µl Standard 5,**
   pipette in positions G1/2 **100 µl Standard 6**,
   pipette in positions H1/2 **100 µl Standard 7.**

   To control correct accomplishment **100 µl** of the **(1:100)** diluted **Control 1** can be pipetted in positions A3/4.
   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** at **room temperature** (shake at 350 rpm).

4) After incubation aspirate the contents of the wells into a disinfectant (risk of infection!) and wash the wells 3 times with **250 µl** of **Washing Buffer** / well respectively. **Washing Buffer** should incubate at least for **15 seconds** / well.

5) Following the last washing step pipette **100 µl** of the of the **(1:100)** diluted **biotin conjugate** in each well, and incubate **1 hour** at **room temperature** (shake at 350 rpm).

6) After incubation wash the wells 3 times with **Washing Buffer** as described above.

7) Following the last washing step pipette **100 µl** of the **(1:100)** diluted **HRP Conjugate** in each well, and incubate **30 min** at **room temperature** (shake at 350 rpm).

8) After incubation wash the wells 3 times with **Washing Buffer** as described above.

9) Pipette **100 µl** of the **Chromogenic substrate** in each well.

10) Incubate the plate for **30 minutes** in the dark at **room temperature**.

11) Stop the reaction by adding **100 µl** of **Stopping Solution** to all wells.

12) Measure the absorbance within **30 minutes** at **450 nm** (reference filter: 620 nm).

EVALUATION OF RESULTS

Establishing the Standard Curve

The standards provided contain the following concentrations of IGFBP-2 :

Standard	A	B	C	D	E	F	G
ng/ml	0.125	0.25	0.5	1	2	4	8

1) Calculate the mean absorbance (MA) value for the blank from the duplicated determination (well A1/A2).

2) Subtract the mean absorbance (MA) of the blank from the mean absorbancies of all other values.

3) Plot the Standard concentrations 1-7 on the x-axis versus the mean value of the absorbancies of the Standards on the y-axis. By using the mean absorbancies of the samples herewith the sample concentrations can be received.

4) Recommendation: Calculation of standard curve and sample concentrations should be done by using a computer programme, because the standard curve is in general best described by a non-linear regression or a higher-grade polynom or four parametric (4PL) curve fits.

5) The mIGFBP-2 concentration of the samples can be calculated with the standard curve equation and by multiplication with the respective dilution factor.

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Summary of the Assay

Reagent preparation:	Reconstitution:	Dilution:
Standards 1-7	in **1 ml Dilution Buffer**	
Control 1	in **100 µl Dilution Buffer**	**1:100** with Dilution Buffer
Biotin conjugate		**1:100** with Dilution Buffer
HRP Conjugate		**1:100** with Dilution Buffer
Washing Buffer		**1:20** with distilled water (e.g., add the complete contents of the flask **(50 ml)** into a graduated flask and fill with distilled water to 1000 ml).
Sample dilution: 1:100 (e.g. Mix 2.5 µl Serum with 247.5 µl Dilution Buffer).		

Assay Procedure for double determination

Pipette	Reagents	Well positions
100 µl	Dilution Buffer (Blank)	A1/2
100 µl	Standard 1 (**0.125 ng/ml**)	B1/2
100 µl	Standard 2 (**0.25 ng/ml**)	C1/2
100 µl	Standard 3 (**0.5 ng/ml**)	D1/2
100 µl	Standard 4 (**1 ng/ml**)	E1/2
100 µl	Standard 5 (**2 ng/ml**)	F1/2
100 µl	Standard 6 (**4 ng/ml**)	G1/2
100 µl	Standard 7 **(8 ng/ml)**	H1/2
100 µl	Control 1	A3/4
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
	Incubation: 1 h at RT, ≥ 350 rpm	
3x 250 µl	Aspirate the contents of the wells and **wash** 3x with 250 µl Washing Buffer	each well
100 µl	1:100 diluted Biotin conjugate	each well
	Incubation: 1 h at RT, ≥350 rpm	
3x 250 µl	Aspirate the contents of the wells and **wash** 3x with 250 µl Washing Buffer	each well
100 µl	1:100 diluted biotin conjugate	each well
	Incubation: 30 min at RT, ≥350 upm	
3x 250 µl	Aspirate the contents of the wells and **wash** 3x with 250 µl Washing Buffer	each well
100 µl	Chromogenic substrate	each well
	Incubation: 30 min in the **dark** at RT	
100 µl	Stopping Solution	each well
Measure the absorbance within 30 min at **450 nm** with **≥590 nm** as reference wavelength.		

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