BioVendor
Research
and Diagnostic Products

Mouse/ Rat IGFBP-3 ELISA

Cat. No.: RMEE031R

TECHNICAL FEATURES + APPLICATIONS

- Quantitative determination of mouse/rat IGFBP-3 without sample pretreatment
- Inter-Assay variation of 8.4% and Intra-Assay variation of 4.6%
- Analytical sensitivity of 0.018 ng/ml (18 pg/ml; 1,8 pg/well)

INTRODUCTION

Growth Hormone, Insulin-like Growth Factors and their binding proteins build up an endocrine system regulating not only longitudinal growth in humans but also influencing a broad variety of other physiological and pathophysiological processes like energy metabolism or tumor growth. Most effects of Growth Hormone (GH) are exerted by Insulin-like Growth Factors (IGF) mainly produced by the liver but also locally by specific tissues. The effects of IGF are also regulated. Specific binding proteins (IGFBP 1-7) regulate bioavailability of IGF. After proteolytic cleavage of the binding proteins IGF is set free and able to bind to its receptor. The autophosphorylation of this thyrosine kinase receptor activates intra cellular signalling cascades. Some of these IGFBPs not only regulate the availability of IGF but also exert IGF-independent effects on cell physiology.

IGFBP-3 is the most abundant IGFBP in circulation and therefore of special relevance in regulation of IGF effects. This is reflected by the indicative value of serum IGFBP-3 concentration in diagnostics of growth disturbances. IGFBP-3 has also been shown to be able to induce apoptosis, promote tumor growth and inhibit cellular migration and metastasis dependent on tissue and tumor stage.

INTENDED USE

This enzyme immunoassay kit is suited for measuring IGFBP-3 in mouse and rat serum, cell lysates or nuclear extracts.

PERFORMANCE CHARACTERISTICS AND VALIDATION

The ELISA for mouse/rat IGFBP-3 (m/rIGFBP-3) RMEE031R is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The IGFBP3 in the sample binds to the immobilized first antibody on the microtiter plate. In the following step, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-mouse IGFBP-3-Antibody binds in turn to the immobilised mIGFBP-3. In the final substrate reaction, the resulting product changes color and is measured spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of m/r IGFBP-3 levels in the samples.

The standards of the ELISA RMEE031R are **recombinant mouse IGFBP-3** in concentrations of **0.078, 0.156, 0.313, 0.625, 1.25, 2.50 ng/ml and 5 ng/ml.**

Sensitivity

The **analytical sensitivity** of the ELISA RMEE031R yields 0.018 **ng/ml** (2 SD of zero standard in 21-fold determination).

The **Inter**- and **Intra-Assay** variation coefficients were found less than **8.36 %** and **4.36%.** Exemplary determinations are shown in table 1 and table 2.

Table 1: Inter-Assay-Variation (n=10)

	Mean Value	Standard Deviation	VC
Sample 1	152.9	6.39	8.36
Sample 2	328.6	5.98	3.64

Table 2: Intra-Assay-Variation (n=16)

	Mean Value	VC
Sample 1	123.8	4.63
Sample 2	395.0	1.99

Cross reactivity with recombinant human IGFBP3: 0.03%

The recovery of rec. mouse IGFBP-3 in **cell culture medium** DMEM was found to be 89.4%, and, in DMEM incl. 5% FCS 92.6%.Therefore cell culture medium seems to be suitable as sample matrix.

Table 3: Linearity (results of 2 different mouse sera)

Dilution:	**Sample 1** (recalculated, ng/ml)	**Dilution:**	**Sample 2** (recalculated, ng/ml)
1:100	351.8	1:20	367.6
1:200	369.1	1:40	414.5
1:400	384.5	1:80	423.4
1:800	381.3	1:160	411.0
1:1600	379.2	1:320	421.9
1:3200	386.1	1:640	455.7
AV / 1SD / VC%	375.3 / 12.9 / 3.46	**AV / 1SD / VC%**	415.7 / 28.4 / 6.83

AV = Average Value , SD = Standard Deviation; VC = Coefficient of Variation

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

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 m/rIGFBP-3 levels were found to be unaffected by few cycles (5x) in our experiments.

The high sensitivity of the assays allows m/rIGFBP-3 determinations in small sample volumes, which is limited by pipetting accuracy rather than the amount of m/rIGFBP-3.

In most determinations (e.g. Serum- or Plasma samples and no extreme values expected) the dilution of **1:301 with Dilution Buffer VP is suitable**, the respective covered range would be 23.5 to 1505 µg/L. Where required, depending on the expected mIGFBP-3-values, the dilution with **Dilution Buffer VP** can be higher or lower.

Suggestion for dilution protocol:

Pipette **1.5 ml Dilution Buffer VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **5 µl Serum**- or **Plasma** (dilution 1:301) and mix each tube **immediately**. After mixing use **100 µl** of this solution within 1 hour **per determination** in the assay.

REAGENT PROVIDED

1)	MTP	**Microtiter plate,** ready for use: Microtiter plate with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with anti-mouse/rat IGFBP-3 Antibody, packed in a laminate bag.
2)	CAL	**Standards A-G**, lyophilised, contain recombinant mouse IGFBP-3. Standard values are between 0,078 – 5 ng/ml (0.078, 0.156, 0.313, 0.625, 1.25 2.5 and 5 ng/ml) mIGFBP-3, Standards are **reconstituted with 750 µl Verdünnungs Buffer VP each**. Use 100 µl pro well in the assay.
3)	BUF X	**Dilution Buffer VP**, 125 ml, ready for use, please use for dilution of **Standards ,Controls** and **Samples**
4)	Controls	**Control Sera KS1 and KS2**, 100 µl, lyophilised. **KS1** contains mouse serum and **KS2** rat serum. Please reconstitute **in 100 µl Dilution Buffer VP**. The m/rIGFBP-3 target values and the respective ranges are given on the label of the vial. The dilution should be according to the dilution of the respective samples.
5)	Ab	**Antibody Conjugate AK**, 12 ml, contains biotinylated anti-mouse IGFBP-3 antibody, ready for use. Pipette 100 µl per well.
6)	CONJ	**Enzyme Conjugate EK**, 12 ml, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin, ready for use. Pipette 100 µl per well.
7)	WASHBUF 20x	**Washing Buffer (WP),** 50 ml, 20 X concentrated solution. Dilute 1:20 with Aqua dest. The 1:20 diluted Washing Buffer **WP** is only limited stable. Please dilute only according to daily requirements.
8)	SUBST	**Substrate (S),** 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
9)	H_2SO_4	**Stopping Solution (SL),** 12 ml, ready for use, 0.2 M sulphuric acid, Caution acid!
10)		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes (100 and 200µl) Micropipettes and multichannel pipettes with disposable plastic tips

Distilled or Deionized water for dilution of the Washing Buffer (WP)

Vortex-mixer

Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human samples)

Timer (120 min. range)

Reservoirs (disposable)

Plate washer and plate shaker (recommended)

Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620nm (or ≥590 nm)

Foil welding device for laminate bags (recommended)

REAGENT PREPARATION

In conducting the assay, follow strictly the test protocol. Room temperature incubation means: Incubation at 20 - 25°C.

Reagents with different lot numbers should not be mixed. The microtiter plate and all reagents are stable unopened until the expiry date, if stored in the dark at 2° - 8°C (see label).

The Standards **A – G** and **Control Sera KS1** and **KS2** are reconstituted with the **Dilution Buffer VP** provided in the Kit. It is recommended to keep the 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 A – G** and **Control Sera KS1 and KS2**) should be stored at -20°C (or below). Freezing extends the expiry at least 2 months. When using the standards anew, please thaw them rapidly but gently (no temperature rise over the room temperature and no powerful vortexing), 3 of these freezing-thawing cycles showed no influence on the assay.

The 1:20 diluted Washing Buffer **WP** is only limited stable. Please dilute only according to daily requirements.
Before use, all kit components should be brought to room temperature.

Precipitates, possible in buffers, should be dissolved before use through mixing and warming.

The **Substrate Solution S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

When performing the assay, the Standards **A-G,** Control Sera and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times the **AK** Antibody Conjugate and Enzyme Conjugate **EK** and 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. **Stop Solution SL** should be added to the plate in the same order as the Substrate Solution **S**.

STORAGE, CONDITIONS

The microtiter plate wells and all undiluted reagents are stable until the expiry date if stored in the dark at 2-8°C. Store the unused seal strips and microtiter wells together with the desiccant at 2° to 8°C.

The Substrate Solution (S), stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

Reconstituted Components (**Standards A – G** and **Control Sera**) should be stored 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 determinations over a longer period with one kit, you should aliquot the components prior to freezing into suitable smaller volumes. This is strongly recommended.

WARNINGS AND PRECAUTIONS

For in-vitro use only. For professional use only.
Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming. **Temperature WILL affect the absorbance** readings of the assay. However, values for the patient samples will not be affected.

Do not mix reagents of different lots. Do not use expired reagents.

The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.

Caution: This kit contains material of human and/or animal origin. Source human serum for the Control Serum provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibody. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

R36/38 Irritating to eyes and skin

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S28.1 After contact with skin, wash immediately with plenty of water

S36/37 Wear suitable protective clothing and gloves.

Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

2-Methyl-4-Isothiazolin-3-one

contained in following components: **AK, EK, VP**

< 0,01% 2-Methyl-4-isothiazolin-3-one Solution

R34 Irritating to eyes and skin

R43 Sensibilisation through skin contact possible

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S36/37 Wear suitable protective clothing and gloves

S45 In case of accident or if you feel unwell seek medical advice

5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one

contained in following components: **AK, EK, VP, WP**

< 0,01% (w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one

Solution

R36/38 Irritating to eyes and skin

R43 Sensibilisation through skin contact possible

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S28.1 S28.1 After contact with skin, wash immediately with plenty of water

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

General 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.

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.

ASSAY PROCEDURE

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

When performing the assay, the Standards, Control Serum 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 **Enzyme –Conjugate EK** as well as the following **Substrate Solution 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 the Substrate Solution.

1) Add **100µl Dilution Buffer VP** in positions A1/2
2) Pipette in positions B1/2 **100µl** each **Standard A (0.078 ng/ml)**,
   pipette in positions C1/2 **100µl** each **Standard B (0.156 ng/ml)**,
   pipette in positions D1/2 **100µl** each **Standard C (0.313 ng/ml)**,
   pipette in positions E1/2 **100µl** each **Standard D (0.625 ng/ml)**,
   pipette in positions F1/2 **100µl** each **Standard E (1.25 ng/ml)**,
   pipette in positions G1/2 **100µl** each **Standard F (2.5 ng/ml)**,

pipette in positions H1/2 **100µl** each **Standard G (5 ng/ml)**,

To control the correct accomplishment **100 µl** of the 1:301 (or in respective dilution rate of the sample) in Dilution Buffer **VP** diluted **Control Sera KS1 and KS2** can be pipetted in positions A3/4 and B3/4.

Pipette **100 µl each** of the **diluted sample** (generally 1:301 diluted in Dilution Buffer **VP**) in the rest of the wells, according to requirements. Please mix the dilutions immediately after sample addition and use within 60 minutes.

3) Cover the wells with the sealing tape and incubate the plate for **1 hour** at **room temperature** (if possible, shake at ≥350 rpm).
4) After incubation aspirate the contents of the wells and wash the wells 3 times with **250 µl Washing Buffer WP**.
5) Following the last washing step, pipette **100 µl** of the **Antibody Conjugate AK.** Cover the wells with the sealing tape and incubate **1 hour** at **room temperature** (if possible shake at ≥350 rpm).
6) After incubation wash the wells 3 times with **Washing Buffer WP** as described in step 4)
7) Following the last washing step, pipette **100 µl** of the **Enzyme Conjugate EK.** Cover the wells with the sealing tape and incubate **15 min** at **room temperature** (if possible shake at ≥350 rpm).
8) After incubation aspirate the contents of the wells and wash the wells 3 times with **250 µl Washing Buffer WP**.
9) Pipette **100 µl of the TMB-Substrate solution S** in each well.
10) Incubate the plate for **15 Minutes in the dark at** room **temperature**.
11) After incubation pipette **100 µl Stop Solution SL** in each well.
12) Measure the absorbance **within 30 minutes at 450 nm** (Reference filter ≥590 nm, e.g. 620 nm).

CALCULATION OF RESULTS

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.20, and the absorbance of standard G should be greater than 1.00.

Samples, which yield higher absorbance values than **Standard G**, are beyond the standard curve, for reliable determinations such samples should be retested at a higher dilution.

Establishing the Standard Curve

The standards provided contain the following concentration of **mIGFBP-3**:

Standard	**A**	**B**	**C**	**D**	**E**	**F**	**G**
ng/ml	0.078	0.156	0.313	0.625	1.25	2.5	5

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 absorbances of all other values.
3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis on semi-log paper (lin-log).
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. **Non-linear regression**, a higher-grade polynomial or four parametric logistic (4-PL) lin-log curve fit are suitable for the evaluation. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.
5) The **mIGFBP-3 concentration in ng/ml** of the samples can be **calculated by multiplication with the respective dilution factor**, Division by 1000 converts the values in µg/ml or equal mg/Litre (Example: a measured value was 3 ng/ml, Sample was 1:301 diluted: 3 x 101= 903 ng/ml, or 0.903 µg/ml or 0.903 mg/L, according the requested unit).

REFERENCES

Silha JV, Murphy LJ: Insulin-like growth factor binding proteins in development. Adv Exp Med Biol. 2005;567:55-89

Yakar S, Sun H, Zhao H, Pennisi P, Toyoshima Y, Setser J, Stannard B, Scavo L,
Leroith D: Metabolic effects of IGF-I deficiency: lessons from mouse models. Pediatr Endocrinol Rev. 2005 Sep;3(1):11-19

Silha JV, Murphy LJ: Insights from insulin-like growth factor binding protein transgenic mice. Endocrinology. 2002 Oct;143(10):3711-4.

Firth SM, Baxter RC: Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev. 2002 Dec;23(6):824-854

Schneider MR, Lahm H, Wu M, Hoeflich A, Wolf E: Transgenic mouse models for studying the functions of insulin-like growth factor-binding proteins. FASEB J. 2000 Apr;14(5):629-40

SUMMARY – MOUSE/RAT IGFBP-3 ELISA RMEE031R

Reconstitution/ Dilution of Reagents		
Standards A-G	Reconstitution in **Dilution Buffer VP**	**750 µl each**
Control Sera KS1 and KS2	Reconstitution in **Dilution Buffer VP**	**100 µl**
Washing Buffer WP	dilute in **A. dest.** (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with A.dest. to 1000 ml)	**1:20**
Sample Dilution + Control Serum KS: 1:301 in Dilution Buffer VP mix directly and use within max. 60 min.		
Before assay procedure bring all **reagents** to **room temperature**		

Proposal of Assay Procedure for Double Determination:

Pipette	Reagents	Well Positions
100 µl	Verdünnungspuffer **VP** as Blank	A1 and A2
100 µl	Standard **A** (**0,078 ng/ml**)	B1 and B2
100 µl	Standard **B** (**0,156 ng/ml**)	C1 and C2
100 µl	Standard **C** (**0,313 ng/ml**)	D1 and D2
100 µl	Standard **D** (**0,625 ng/ml**)	E1 and E2
100 µl	Standard **E** (**1,25 ng/ml**)	F1 and F2
100 µl	Standard **F** (**2,5 ng/ml**)	G1 and G2
100 µl	Standard **G** (**5 ng/ml**)	H1 und H2
100 µl	Control Serum **KS1**	A3 und A4
100 µl	Control Serum **KS2**	B3 und B4
10 µl	**Sample**	Pipette sample in the rest of the wells according to requirements
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** each **WP/well**	each well
100 µl	Antibody Conjugate **AK**	each well

Incubation: 1 h at RT, ≥350 rpm

3x 250 µl	Aspirate the contents of the wells and wash **3x** with **250 µl** each **WP/well**	each well
100 µl	Enzyme Conjugate **EK**	each well

Incubation: 15 min at RT, ≥350 rpm

3x 250 µl	Aspirate the contents of the wells and wash **3x** with **250 µl** each **WP/well**	each well

CAL A-G	A-G	Rec in 750 µl VP	100 µl
Control	KS1 and KS2	Rec in 100 µl VP	100 µl
WASHBUF 20x	WP		1:20 DILU A. dest.
SPE + Control 1:301 DILU VP			100 µl
°C 20-25 °C			

100 µl	VP	A1/2
100 µl	CAL A (0.078 ng/ml)	B1/2
100 µl	CAL B (0.156 ng/ml)	C1/2
100 µl	CAL C (0.313 ng/ml)	D1/2
100 µl	CAL D (0.625 ng/ml)	E1/2
100 µl	CAL E (1.25 ng/ml)	F1/2
100 µl	CAL F (2.5 ng/ml)	G1/2
100 µl	CAL G (5 ng/ml)	H1/2
100 µl	CONTROL KS1 1:301 DILU VP	A3/4
100 µl	CONTROL KS2 1:301 DILU VP	B3/4
100 µl	SPE 1:301 DILU VP	
TAPE		

1 h °C 20-25 ↔ ≥ 350 rpm

3x 250 µl	3x WASHBUF WP
100 µl	Ab AK

Distributed by

BioVendor - Laboratorní medicína a.s.
CTPark Modrice
Evropska 873
664 42 Modrice
CZECH REPUBLIC
Phone: +420-549 124 185
Fax: +420-549 211 460
e-mail: info@biovendor.com
http://www.biovendor.com

European Union:

BioVendor GmbH
Im Neuenheimer Feld 583
D-69120 Heidelberg, GERMANY
Phone: +49-6221-433-9100
Fax: +49-6221-433-9111
e mail: roth@biovendor.com
e mail: abels@biovendor.com

China - Hong Kong Office:

BioVendor Laboratories Ltd.
Room 4008, Hong Kong Plaza, No.188
Connaught Road West
Hong Kong, CHINA
Phone: +852 28030523
Fax: +852 28030525
e-mail: lu@biovendor.com

USA, Canada and Mexico:

BioVendor, LLC
1463 Sand Hill Road, Suite 227
Candler, NC 28715, USA
Phone: +1-828-670-7807, +1-800-404-7807
Fax: +1-828-670-7809
e mail: info-us@biovendor.com

China – Mainland Office:

BioVendor Laboratories Ltd.
Room 2405, YiYa Tower, TianYu Garden, No.150
Lihe Zhong Road, Guang Zhou, CHINA
Phone: +86-20-87063029
Fax: +86-20-87063016
e-mail: jhsu@biovendor.com

Manufactured by

Aspenhausttr. 25
D-71770 Reutlingen
Germany
http://www.mediagnost.de
e-mail: contact@mediagnost.de