



Human IGFBP-1 ELISA

Cat. No.: RMEE01



TECHNICAL FEATURES+APPLICATIONS

- ◆ **Quantitative determination** of IGFBP-1 in serum and in other body fluids, like e.g. amniotic fluid, milk, urine or saliva etc. and in cell culture media.
- ◆ extremely high **analytical sensitivity of 0.02 ng/ml**
- ◆ Inter-Assay variation of 7,4% and Intra-Assay variation of 6,8%
- ◆ results available in **only 1.75 h incubation time**

INTRODUCTION

The Insulin-like Growth Factors I and – II are free in body fluids and tissues but are bound to specific binding proteins. Until today seven different binding proteins (IGFBP-1 to –7) can be differentiated additionally several IGFBP-related proteins have also been detected. Bioavailability of IGF is regulated by these IGFBPs or better their proteolytic cleavage which reduces affinity to IGF. But the IGFBPs as well as their proteolytic fragments can also exert IGF-independent effects, like influencing cell migration or proliferation. IGFBP-1 (Placental Protein 12) consists of 234 aminoacids and has a molecular weight of approximately 25kDa. The coding DNA region is located on chromosome 7. IGFBP-1 is mainly synthesized by foetal and adult liver tissue and decidual endometrium. Intensity of Expression varies enduring menstruation with a maximal expression in the late secretory phase. IGFBP-1 is posttranslational modified by phosphorylation of serine residues 101, 119 and 169.

Phosphorylation has physiological relevance as it increases affinity of IGFBP-1 to IGF. In adult humans phosphorylated IGFBP-1 of the liver is the predominant form in circulation. IGFBP-1 produced by endometrial tissue is significantly less phosphorylated than the liver originated form.

INTENDED USE

This enzyme immunoassay kit is suited for measuring IGFBP-1 in human serum or Heparin and EDTA plasma or in other body fluids, for example amnion fluid, mother milk, urine or saliva, as for diagnostic and scientific purposes. It is also suited to quantitate IGFBP-1 in cell culture media.

PERFORMANCE CHARACTERISTICS and Validation

The ELISA for IGFBP-1 RMEE01 is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The IGFBP-1 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-IGFBP-1-Antibody binds in turn to the immobilised IGFBP-1. Finally, the bound peroxidase catalyses the substrate reaction resulting in a colored product. Therefore colour intensity is highly specific and quantitatively depending on the IGFBP-1- level of the samples.

The standards of the ELISA RMEE01 are **native human IGFBP-1** in concentrations of **0; 0.1; 0.5; 1; 2; 4 and 8 ng/ml**.

The **analytical sensitivity** of the ELISA RMEE01 yields **0.02 ng/ml** (equal to **2 pg per well**; 2 SD of zero standard in 22fold determination).

The determination of IGFBP-1 with Mediagnost ELISA RMEE01 is over a very wide range authentic in dilution. The **linearity of serum dilutions** is over a wide range **excellent** (table 1).

Table 1: Linearity of Dilution (typical results of 2 different sera)

Dilution:	sample 1 (re-calculated, ng/ml)	Dilution:	sample 2 (re-calculated, ng/ml)
1:2.5	14.38	1:2.5	16.81
1:5	14.22	1:5	15.51
1:10	13.42	1:10	16.22
1:20	13.81	1:20	14.45
1:40	13.11	1:40	15.12
1 :80	12.52	1 :80	13.43
1 :160	14.65	1 :160	15.95
AV / 1SD / CV%	13.73 / 0.76 / 5.53	AV / 1SD / CV%	15.36 / 1.14 / 7.44

AV = average value, SD = standard deviation, CV = coefficient of variation

The **recovery** of native IGFBP-1 in different sample matrices is listed in table 4 (page 13). The measured **cross reactivity** for recombinant IGFBP-2 as well as IGFBP-3 was found to be negligible. 500 ng/ml recombinant material was diluted in buffer and this sample applied to the test system. Measured crossreactivity was **less than 0.0015%** in both cases.

The **Inter-** and **Intra-Assay** coefficients of variation were found to be less than **7.4% and 6.8%**. Exemplary determinations are shown in table 2 and table 3.

Table 2: Inter-Assay-Variation

	Average Value (ng/ml)	Standard Deviation (ng/ml)	Coefficient of Variation (%)
Sample 1	2.31	0.12	5.23
Sample 2	18.41	1.36	7.36
Sample 3	32.79	2.22	6.75

Table 3: Intra-Assay-Variation

	Average Value (ng/ml)	Standard Deviation (ng/ml)	Coefficient of Variation (%)
Sample 1	1.45	0.08	5.87
Sample 2	20.64	1.29	6.23
Sample 3	162.99	11.09	6.81

The comparison of IGFBP-1 determinations of 35 sera from healthy adults with the Mediagnost ELISA RMEE01 and another commercially available ELISA yields a very good accordance of absolute concentrations by a **very high correlation**: $y = 1.15x + 0.12$; $r^2 = 0.94$, the comparison with a further commercial ELISA yields, at a likewise **very high correlation**: $y = 3.33x + 3.0$; $r^2 = 0.90$, measured values of approx. one third of the respective concentrations.

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum samples, EDTA- and Heparin-Plasma samples are suitable. A special external sample preparation prior to assay is not required. Results in Citrate-Plasma are about 15% reduced. Slight hemolysis of the samples doesn't disturb the determination. 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 IGFBP-1 levels were found to be unaffected by few cycles(3x) in our experiments.

In most determinations (e.g. Serum- or Plasma samples and no extreme values expected, see table 4 for further details) the dilution of **1:16 with Dilution Buffer VP is suitable**, the respective covered range would be 0 to 128 ng/ml.

Suggestion for dilution protocol:

Pipette 300 µl **Dilution Buffer VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **20 µl Serum- or Plasma** (dilution 1:16) and mix each tube **immediately**. After mixing use **50 µl** of this solution within 1 hour **per determination** in the assay.

Where required, depending on the expected IGFBP-1-values, the dilution with Dilution Buffer VP can be higher or lower (at least however 1:2.5). The IGFBP-1 concentrations maybe completely different in body fluids of human origin other than serum or in cell culture supernatants. Examples as well as dilution recommendations are given in table 4.

Table 4: Sample matrices, recovery and dilution recommendation

Samples	Concentration IGFBP-1 (ng/ml)	Recovery of added IGFBP-1	Recommended Dilution as Sample in RMEE01
Amniotic Fluid	8,140.0 16,450.0	n.d.	individually different at least 1:5000 up to 1:25000
Mother Milk	5.12 20.2	91% (at 1:10 dil.) n.d.	1:10
Urine	0.07	89.8% (at 1:2.5 dil.)	1:2.5
Saliva	< 0.02 ng/ml	62.5% (at 1:2.5 dil.)	at least 1:2.5
Bronchial Lavage	< 0.02 ng/ml	100% (at 1:2.5 dil.)	1:2.5
Sputum	< 0.02 ng/ml	100% (at 1:20 dil.)	1:20
Serum pool	0.57	105.1% (at 1:16 dil.)	1:16 (general recommendation)
Pregnancy sera	n.d.	n.d.	1:25
Cell Culture Media	individually different	94.5% (at 1:5 dil.)	individually different at least 1:5

n.d.= not determined

REAGENTS 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-human IGFBP-1 Antibody, packed in a laminate bag.
2)	CAL	Standards A-G , lyophilised, contain native human IGFBP-1. Standard values are between 0 – 8 ng/ml (0; 0.1; 0.5; 1; 2; 4 and 8 ng/ml) IGFBP-1, Standards are reconstituted with 500 µl Dilution Buffer VP each. Use 50 µl pro well in the assay.
3)	BUF X	Dilution Buffer VP , 125 ml, ready for use, please use for dilution of samples, control and standards.
4)	Control	Control Sera KS1 and KS2 , 250 µl, lyophilised, contain human Serum and should be reconstituted in 250 µl Dilution Buffer VP each . The IGFBP-1 target values and the respective ranges are given on the vial label. The dilutions should be according to the dilution of the respected samples. Use 50 µl pro well in the assay.
5)	Ab	Antibody Conjugate AK , 6 ml, contains biotinylated anti-human IGFBP-1 Antibody. Use 50 µl pro well in the assay.
	CONJ	Enzyme Conjugate EK , 12 ml, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin. Ready for use. Use 100 µl pro well in the assay.
6)	WASHBUF 20x	Washing Buffer (WP) , 50 ml, 20 X concentrated solution. Dilute 1:20 with Aqua dest. Attention: After dilution the Washing Buffer is only 4 weeks stable, dilute only according to requirements.
7)	SUBST	Substrate (S) , 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H ₂ O ₂ Tetramethylbencidine. Use 100 µl pro well in the assay.
8)	H ₂ SO ₄	Stopping Solution (SL) , 12 ml, ready for use, 0.2 M sulphuric acid, Caution acid! Use 100 µl pro well in the assay
9)		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes (100 and 200 µl) 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)
Microtiter Plate washer (recommended)
Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620 nm (or ≥ 590 nm)
Foil welding device for laminate bags (recommended)
Polyethylen PE/Polypropylen PP tubes for dilution of samples

REAGENT PREPARATION

The assay has to be conducted strictly according the test protocol herein.

The danger of handling with potentially infectious material must be taken into account.

Reagents with different lot numbers cannot be mixed. The microtiterplate and reagents are stable until the indicated expiry, if stored unopened and protected from sunlight at 2 – 8°C.

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.

Incubation at room temperature means: 20-25°C

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 diluted to usage concentration. Washing volume per washing cycle and well must be 300 µl at least.

When using an automatic microtitre 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. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamically swinging out the microtitre 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.

Standards and Controls

For the reconstitution of the lyophilised components (Standards A - G and Control Sera KS1 & KS2) the kit **Dilution Buffer VP** has to be used. 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 reconstituted standards and controls can be stored for 3 months at -20°C . Repeated freeze/thaw cycles have to be avoided. 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. 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.

Washing Buffer

The required volume of washing buffer is prepared by 1:20 dilution of the provided 20-fold concentrate with deionised water. The diluted Washing Buffer is stable for max. 4 weeks at $2-8^{\circ}\text{C}$.

Substrate Solution

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

Microtiterplate

Store the once unused microtiter strips and wells together with the desiccant in the tightly closed clip lock bag at $2-8^{\circ}\text{C}$ use in the frame provided. The labelled expiry is not influenced in case of proper storage.

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic 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. Be sure that everything is understood. The Mediagnost GmbH is not liable for any loss or harm caused by non-observance of the instructions, as far as no law withstands.

Temperature WILL affect the absorbance readings of the assay. However, values for the patient samples will not be affected.

Do not use expired reagents.

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.

Caution: This kit contains material of human and/or animal origin.

Human Serum

Contained in following components: **Control Serum KS1 and KS2.**

The sources of human sera were 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) antibodies. 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 (H₂SO₄)

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

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 After contact with skin, wash immediately with plenty of water

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 Sera 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 Sera and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, the **Enzyme Conjugate EK**, the **Substrate Solution S** as well as the **Stop Solution SL** should be added to the plate in the same order and in the same time interval each, respectively.

IMPORTANT: Please leave the wells A1/A2 until addition of the **Substrate Solution**, step 8, empty.

1) Please pipette in all needed wells, **except A1/A2, 50 µl Antibody Conjugate AK.**

- 2) Pipette in positions B1/2 **50 µl each Standard A (0 ng/ml)**,
pipette in positions C1/2 **50 µl each Standard B (0.1 ng/ml)**,
pipette in positions D1/2 **50 µl each Standard C (0.5 ng/ml)**,
pipette in positions E1/2 **50 µl each Standard D (1 ng/ml)**,
pipette in positions F1/2 **50 µl each Standard E (2 ng/ml)**,
pipette in positions G1/2 **50 µl each Standard F (4 ng/ml)**,
pipette in positions H1/2 **50 µl each Standard G (8 ng/ml).**

To control the correct accomplishment, **50 µl** of the **1:16** (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 **50 µl each** of the **diluted samples** (generally 1:16 diluted in Dilution Buffer **VP**, please mix the dilutions immediately after sample addition and use within 60 minutes) in the rest of the wells, according to requirements.

- 3) Cover the wells with the sealing tape and incubate the plate for **1 hour at room temperature**
- 4) After incubation aspirate the contents of the wells and wash the wells **5 times** with **300 µl Washing Buffer WP**.
- 5) Following the last washing step, pipette **100 µl Enzyme Conjugate EK** in each well, **except A1/A2**.
- 6) Cover the wells with the sealing tape and incubate **30 min at room temperature**
- 7) After incubation wash the wells 5 times with **Washing Buffer WP** as described in step 4)
- 8) Pipette **100 µl of the TMB-Substrate solution S** in each well, **also in A1/A2**.
- 9) Incubate the plate for **15 Minutes in the dark at room temperature**.
- 10) After incubation pipette **100 µl Stop Solution SL** in each well, **also in A1/A2**.
- 11) Measure the absorbance **within 30 minutes at 450 nm (Reference filter ≥590 nm)**.

CALCULATION OF RESULTS

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**, 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 native hIGFBP-1:

Standard	A	B	C	D	E	F	G
ng/ml	0	0.1	0.5	1	2	4	8

- 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 absorbances of all other values.
- 3) Plot the standard concentrations on the x-axis versus the mean value of the absorbances 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 **concentration in ng/ml** of the samples can be calculated **by multiplication** with the respective dilution factor.

EXPECTATION VALUES

Concentrations of IGFBP-1 in human sera of 69 healthy adult donors were determined with the Mediagnost ELISA RMEE01. Slight gender dependent differences were found, the concentrations of all samples varied from minimal 0.23 ng/ml to maximal 17.94 ng/ml (see table 5).

Table 5: Expectation values in sera of healthy adults (measured values in ng/ml)

Gender	No. of Samples	Average value	Median	Min. – Max.:
female	33	4.79	4.24	0.23 – 16.07
male	36	5.22	2.71	0.42 – 17.94
total	69	5.01	2,77	0.23 – 17.94

SUMMARY – IGFBP-1 ELISA RMEE01R

Reconstitution / Dilution of Reagents		
Standards A-G	Reconstitution in 500 µl Dilution Buffer VP	
Control Sera KS1 and KS2	Reconstitution in 250 µl Dilution Buffer VP	
Wash Buffer WP	dilute in A. dest. (eg. total volume of 50 ml in a graduated flask and fill up to 1000 ml)	1:20
Dilute Sample and Control Sera KS1 and KS2 1:16 with Dilution Buffer DB		
Before beginning the test procedure bring all reagents to room temperature.		

Assay Procedure for Double Determinations:

Pipette	Reagent	Position
IMPORTANT: Leave the position A1 / A2 empty until addition of Substrate		
50 µl	Antibody Conjugate AK	In all wells except A1 / A2
50 µl	Standard A (0 ng/ml)	B1 and B2
50 µl	Standard B (0.1 ng/ml)	C1 and C2
50 µl	Standard C (0.5 ng/ml)	D1 and D2
50 µl	Standard D (1 ng/ml)	E1 and E2
50 µl	Standard E (2 ng/ml)	F1 and F2
50 µl	Standard F (4 ng/ml)	G1 and G2
50 µl	Standard G (8 ng/ml)	H1 and H2
50 µl	1:16 diluted Control Serum KS1	A3 and A4
50 µl	1:16 diluted Control Serum KS2	B3 and B4
50 µl	1:16 diluted Samples	following wells
Cover the wells with the sealing tape.		

Incubation: 1 h at RT, without shaking

5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl Wash Buffer WP	each well
100 µl	Enzyme Conjugate EK	each well, except A1/A2

Incubation: 30 min at RT, without shaking

5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl Wash Buffer WP	each well
100 µl	Substrate S	each well

Incubation: 15 min in the dark RT

100 µl	Stop Solution SL	each well
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.		



CAL	A-G	A -G	Rec in 500 µl VP	500 µl
Control		KS1, KS2	Rec in 250 µl VP	250 µl
WASHBUF	20x	WP		1:20 DILU A. dest.

SPE + Control	1:16	DILU VP	50 µl
°C	20-25 °C		

50 µl	AK	B1/2 → End
50 µl	CAL A (0 ng/ml)	B1/2
50 µl	CAL B (0.1 ng/ml)	C1/2
50 µl	CAL C (0.5 ng/ml)	D1/2
50 µl	CAL D (1 ng/ml)	E1/2
50 µl	CAL E (2 ng/ml)	F1/2
50 µl	CAL F (4 ng/ml)	G1/2
50 µl	CAL G (8 ng/ml)	H1/2
50 µl	CONTROL KS1 1:16 ↔	A3/4
50 µl	CONTROL KS2 1:16 ↔	B3/4
50 µl	SPE 1:16 DILU VP (20 µl SPE +300 µl VP) ↔	
TAPE		

1 h **°C** 20-25

5x 300 µl	5x WASHBUF WP	
100 µl	CONJ EK	B1/2→ End
TAPE		

0.5 h **°C** 20-25

5x 300 µl	5x WASHBUF WP	
100 µl	SUBST TMB S	A1/2→ End

0.25 h **°C** 20-25



100 µl	H₂SO₄ SL	A1/2→ End
MEASURE		

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