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 [1, 2]. 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 [3, 4]. Further IGFBP-1 expression seems to be regulated by Insulin concentration, with Insulin inhibiting the expression. Insulin regulation results in diurnal fluctuations of up to factor 10 [5].

IGFBP-1 is posttranslational modifed 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 [6].
In pregnancy IGFBP-1 maternal serum concentration increases significantly with maximal values in the second trimester or 22-23 week of gestation (75.8 ng/ml) [5] and decreases slowly until term. IGFBP-1 concentration are not only increased in maternal but also in foetal serum and with extremely high concentrations in amnion fluid. Here concentration can reach more than the 1000-fold of serum values [7]. Long-term changes of serum IGFBP-1 concentration can also be found in amnion fluid: IGFBP-1 level of the child decreases after birth until it reaches the low steady-state level of puberty and adulthood [8, 9].

Short term IGFBP-1 serum concentration is strongly influenced by nutrition level and therewith by insulin. Decreasing IGFBP-1 levels can be found enduring fasting or in diabetes; IGFBP-1 levels increase in case of intensive exercises [10-12].

Relevance of serum and amnion IGFBP-1 in diagnostics has been investigated in several areas. A diagnostic value was assigned for trisomy 18, intrauterine growth retardation, endometrial tumors and pre-eclampsia [14]. Thoroughly investigated was the diagnostic value in insulin resistance and pre-term rupture of the membrane and especially in the second field a significant diagnostic value could be demonstrated.

**Energy metabolism**

Based on the influence of Insulin on IGFBP-1 serum concentrations IGFBP-1 is said to be a possible marker for insulin resistance. Because measurement of IGFBP-1 is much easier facilitated than Glucose – uptake rate this would simplify diagnosis of insulin resistance.

In a small study Maddux et al were able to demonstrate with 23 non-diabetic patients, that IGFBP-1 serum concentration correlated very well with Glucose-uptake rate, even better than the HOMA index does [13].

**Pregnancy**

In pregnancy a significant difference in IGFBP-1 serum concentration of healthy pregnant and diabetic and pre-eclamptic women was found (102,8 vs. 203,71 or 281,09 ng/ml respectively) [15]. Also the evaluation of IGFBP-1 as marker for membrane rupture showed a high specificity (97%) and sensitivity (75%) of IGFBP-1 in vaginal/cervical secrets. In case of intact membrane IGFBP-1 concentration was < 90 ng/ml in the secretion. Enduring 8 hours after spontaneous or induced membrane rupture IGFBP-1 values increased significantly with a median concentration of 1900 ng/ml. In this study IGFBP-1 concentrations von >100ng/ml were set as threshold for detection of amnion fluid and therewith diagnosis of membrane rupture [16]. A positive predictive value of 97% clearly shows that IGFBP-1 is a suitable marker for premature membrane rupture [17].

**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 Mediagnost ELISA for IGFBP-1 E01 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 E01 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 E01 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 E01 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)

<table>
<thead>
<tr>
<th>Dilution:</th>
<th>sample 1 (re-calculated, ng/ml)</th>
<th>Dilution:</th>
<th>sample 2 (re-calculated, ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2.5</td>
<td>14.38</td>
<td>1:2.5</td>
<td>16.81</td>
</tr>
<tr>
<td>1:5</td>
<td>14.22</td>
<td>1:5</td>
<td>15.51</td>
</tr>
<tr>
<td>1:10</td>
<td>13.42</td>
<td>1:10</td>
<td>16.22</td>
</tr>
<tr>
<td>1:20</td>
<td>13.81</td>
<td>1:20</td>
<td>14.45</td>
</tr>
<tr>
<td>1:40</td>
<td>13.11</td>
<td>1:40</td>
<td>15.12</td>
</tr>
<tr>
<td>1:80</td>
<td>12.52</td>
<td>1:80</td>
<td>13.43</td>
</tr>
<tr>
<td>1:160</td>
<td>14.65</td>
<td>1:160</td>
<td>15.95</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Average Value (ng/ml)</th>
<th>Standard Deviation (ng/ml)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>2.31</td>
<td>0.12</td>
<td>5.23</td>
</tr>
<tr>
<td>Sample 2</td>
<td>18.41</td>
<td>1.36</td>
<td>7.36</td>
</tr>
<tr>
<td>Sample 3</td>
<td>32.79</td>
<td>2.22</td>
<td>6.75</td>
</tr>
</tbody>
</table>
Table 3: Intra-Assay-Variation

<table>
<thead>
<tr>
<th></th>
<th>Average Value (ng/ml)</th>
<th>Standard Deviation (ng/ml)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.45</td>
<td>0.08</td>
<td>5.87</td>
</tr>
<tr>
<td>Sample 2</td>
<td>20.64</td>
<td>1.29</td>
<td>6.23</td>
</tr>
<tr>
<td>Sample 3</td>
<td>162.99</td>
<td>11.09</td>
<td>6.81</td>
</tr>
</tbody>
</table>

The comparison of IGFBP-1 determinations of 35 sera from healthy adults with the Mediagnost ELISA E01 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 sub aliquote) 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

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

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.

6) **CONJ**  
Enzyme Conjugate EK, 12 ml, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin. Ready for use. Use 100 µl pro well in the assay.

7) **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.

8) **SUBST**  
Substrate (S), 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H2O2-Tetramethylbencidine. Use 100 µl pro well in the assay.

9) **H2SO4**  
Stopping Solution (SL), 12 ml, ready for use, 0.2 M sulphuric acid, Caution acid! Use 100 µl pro well in the assay.

Sealing tape for covering of the microtiter plate, 2 x, adhesive.
MATERIALS REQUIRED BUT NOT PROVIDED

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

REAGENT PREPARATION

The assay has to be conducted strictly according the test protocol herein. Reagents with different lot numbers cannot be mixed. The microtiter plate and reagents are stable until the indicated expiry, if stored unopened and protected from sunlight at 2 – 8°C.

The shelf life of the components after opening is not affected, if used appropriately. 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.

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

Substrate Solution
The Substrate Solution S, stabilised H2O2-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°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 pipetted 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:

<table>
<thead>
<tr>
<th>Standard</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td>0</td>
<td>0.1</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

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 Medig nost ELISA E01. 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>
<thead>
<tr>
<th>Gender</th>
<th>No. of Samples</th>
<th>Average value</th>
<th>Median</th>
<th>Min. – Max.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td>33</td>
<td>4.79</td>
<td>4.24</td>
<td>0.23 – 16.07</td>
</tr>
<tr>
<td>male</td>
<td>36</td>
<td>5.22</td>
<td>2.71</td>
<td>0.42 – 17.94</td>
</tr>
<tr>
<td>total</td>
<td>69</td>
<td>5.01</td>
<td>2.77</td>
<td>0.23 – 17.94</td>
</tr>
</tbody>
</table>
SUMMARY – IGFBP-1 ELISA RMEE01R

Reconstitution / Dilution of Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Buffer</th>
<th>Volume</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards A-G</td>
<td></td>
<td>500 µl Dilution Buffer VP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Sera KS1 and KS2</td>
<td></td>
<td>250 µl Dilution Buffer VP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash Buffer WP</td>
<td>dilute in A. dest. (eg. total volume of 50 ml in a graduated flask and fill up to 1000 ml)</td>
<td>1:20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Reagent</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µl</td>
<td>Antibody Conjugate AK</td>
<td>In all wells except A1 / A2</td>
</tr>
<tr>
<td>50 µl</td>
<td>Standard A (0 ng/ml)</td>
<td>B1 and B2</td>
</tr>
<tr>
<td>50 µl</td>
<td>Standard B (0.1 ng/ml)</td>
<td>C1 and C2</td>
</tr>
<tr>
<td>50 µl</td>
<td>Standard C (0.5 ng/ml)</td>
<td>D1 and D2</td>
</tr>
<tr>
<td>50 µl</td>
<td>Standard D (1 ng/ml)</td>
<td>E1 and E2</td>
</tr>
<tr>
<td>50 µl</td>
<td>Standard E (2 ng/ml)</td>
<td>F1 and F2</td>
</tr>
<tr>
<td>50 µl</td>
<td>Standard F (4 ng/ml)</td>
<td>G1 and G2</td>
</tr>
<tr>
<td>50 µl</td>
<td>Standard G (8 ng/ml)</td>
<td>H1 and H2</td>
</tr>
<tr>
<td>50 µl</td>
<td>1:16 diluted Control Serum KS1</td>
<td>A3 and A4</td>
</tr>
<tr>
<td>50 µl</td>
<td>1:16 diluted Control Serum KS2</td>
<td>B3 and B4</td>
</tr>
<tr>
<td>50 µl</td>
<td>1:16 diluted Samples</td>
<td>following wells</td>
</tr>
</tbody>
</table>

Cover the wells with the sealing tape.

Incubation: 1 h at RT, without shaking

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Reagent</th>
<th>Quantity</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x 300 µl</td>
<td>Aspirate the contents of the wells and wash</td>
<td>each well</td>
<td></td>
</tr>
<tr>
<td>100 µl</td>
<td>Enzyme Conjugate EK</td>
<td>each well, except A1/A2</td>
<td></td>
</tr>
</tbody>
</table>

Incubation: 30 min at RT, without shaking

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Reagent</th>
<th>Quantity</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x 300 µl</td>
<td>Aspirate the contents of the wells and wash</td>
<td>each well</td>
<td></td>
</tr>
<tr>
<td>100 µl</td>
<td>Substrate S</td>
<td>each well</td>
<td></td>
</tr>
</tbody>
</table>

Incubation: 15 min in the dark RT

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Reagent</th>
<th>Quantity</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µl</td>
<td>Stop Solution SL</td>
<td>each well</td>
<td></td>
</tr>
</tbody>
</table>

Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.
**Human IGFBP-1 ELISA (page 12 of 15)**

**International Test Description**

<table>
<thead>
<tr>
<th>CAL</th>
<th>A-G</th>
<th>A-G</th>
<th>Rec in</th>
<th>500 µl</th>
<th>VP</th>
<th>500 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>KS1, KS2</td>
<td>Rec in</td>
<td>250 µl</td>
<td>VP</td>
<td>250 µl</td>
<td></td>
</tr>
<tr>
<td>WASCHBU</td>
<td>20x</td>
<td>WP</td>
<td>1:20 DILU A. dest.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SPE + Control 1:16 DILU VP 50 µl**

°C 20-25 °C

**50 µl**

- AK
- CAL A (0 ng/ml)
- CAL B (0.1 ng/ml)
- CAL C (0.5 ng/ml)
- CAL D (1 ng/ml)
- CAL E (2 ng/ml)
- CAL F (4 ng/ml)
- CAL G (8 ng/ml)
- CONTROL KS1 1:16
- CONTROL KS2 1:16
- SPE 1:16 DILU VP (20 µl SPE + 300 µl VP)

**TAPE**

°C 1 h 20-25

- 5x 300 µl
- 5x WASCHBU WP
- 100 µl CONJ EK B1/2 → End

**TAPE**

°C 0.5 h 20-25

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

**TAPE**

°C 0.25 h 20-25

- 100 µl H₂SO₄ SL A1/2 → End

**MEASURE**

**REF RME E01**
REFERENCE

