IGFBP-3 RIA-CT

Radioimmunoassay with Coated Tubes for Quantitative Measurement of

Insulin-like Growth Factor Binding Protein-3

Product Code: IGF-R11

100 tubes

For In-Vitro Use Only!

Aspenhaustr. 25 • D-72770 Reutlingen / Germany
Phone: + 49 - (0) 7121 51484-0 • Fax: + 49 - (0) 7121 51484-10
E-mail: contact@mediagnost.de • http://www.mediagnost.de
# Table of the contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEATURES</td>
<td>3</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>3</td>
</tr>
<tr>
<td>INTENDED USE</td>
<td>7</td>
</tr>
<tr>
<td>PRECAUTIONS</td>
<td>8</td>
</tr>
<tr>
<td>General</td>
<td>8</td>
</tr>
<tr>
<td>Radioactivity</td>
<td>9</td>
</tr>
<tr>
<td>METHODOLOGY</td>
<td>10</td>
</tr>
<tr>
<td>Assay Characteristics and Validation</td>
<td>10</td>
</tr>
<tr>
<td>Clinical Validation</td>
<td>11</td>
</tr>
<tr>
<td>Sample Handling and Storage</td>
<td>11</td>
</tr>
<tr>
<td>MATERIALS</td>
<td>12</td>
</tr>
<tr>
<td>Materials Provided</td>
<td>12</td>
</tr>
<tr>
<td>Required Materials Not Provided</td>
<td>13</td>
</tr>
<tr>
<td>Reagent Storage and Preparation</td>
<td>13</td>
</tr>
<tr>
<td>Sample Preparation</td>
<td>14</td>
</tr>
<tr>
<td>Simultaneous IGF-I determination:</td>
<td>14</td>
</tr>
<tr>
<td>ASSAY PROCEDURE</td>
<td>15</td>
</tr>
<tr>
<td>EVALUATION OF RESULTS</td>
<td>17</td>
</tr>
<tr>
<td>Establishing the Standard Curve:</td>
<td>17</td>
</tr>
<tr>
<td>EVALUATION OF SAMPLE CONCENTRATIONS:</td>
<td>18</td>
</tr>
<tr>
<td>Concentration of control:</td>
<td>18</td>
</tr>
<tr>
<td>EXPECTED VALUES</td>
<td>19</td>
</tr>
<tr>
<td>LIMITATONS</td>
<td>19</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>21</td>
</tr>
<tr>
<td>SUMMARY OF THE ASSAY</td>
<td>24</td>
</tr>
</tbody>
</table>
FEATURES

- Quantitative determination of Insulin-like Growth Factor Binding Protein-3 (IGFBP-3)
- Measures growth hormone (GH) dependent IGFBP-3 for evaluating growth disorders
- Stable plasma levels due to absence of circadian variation
- Integrates the GH secretory state over days
- A single measurement is highly informative for diagnosis of GH deficiency or GH excess
- Superior to IGF-I measurement for diagnosis of GH deficiency in young children
- Small sample requirement, thus ideal for pediatric patients
- Easy Handling:
  - Separation by means of specifically coated tubes
  - No time-consuming centrifugation required
  - More reliable performance by means of coloured solutions

INTRODUCTION

Insulin-like growth factors (IGF)-I and -II are bound to specific binding proteins (IGFBPs) in the circulation. To date, at least six binding proteins can be distinguished on the basis of their amino acid sequence. They are designated as IGFBP-1, IGFBP-2, ..., IGFBP-6 (1). Lately the discovery of a new IGFBP-7 has been discussed (2). The predominating IGFBP in blood is IGFBP-3 which largely determines the total IGF-I and IGF-II concentration. In contrast to the other binding proteins, IGFBP-3 has the unique property to associate with an acid-labile non-binding subunit (ALS) after binding of either IGF-I or IGF-II (3-5).
Most of the IGFBP-3 in plasma is present as the high molecular weight ternary complex, however, small amounts of free IGFBP-3 are also found (6,7).

The development of specific radioimmunoassays for IGFBP-3, that also recognize the complete high molecular weight complex, provided new in-sights into its regulation (6-9). On the basis of these findings serum IGFBP-3 has proved to be an additional useful test in the repertoire of diagnostic tools for evaluation of growth disorders (7,8).

Several factors besides GH influence IGFBP-3 levels: age including sexual development, nutrition, hypothyroidism, diabetes mellitus, liver function and kidney function. IGFBP-3 levels are decreased by malnutrition, although less than IGF-I, in hypothyroidism, in diabetes mellitus and in hepatic failure (6-8), but are increased in chronic renal failure (6,10,11). Measurement over 24 hours revealed constant circadian levels (12,13). For clinical practice, the most important regulatory factor is GH. Single IGFBP-3 measurements correlate significantly with the logarithm of the integrated spontaneous GH secretion (8,14). In patients with GH deficiency, IGFBP-3 levels are subnormal and increase gradually to within the normal range after several days of GH administration (7,8). The slow response to GH and constant circadian levels during chronic daily application of GH (13) suggest that IGFBP-3 reflects the GH secretory state over days.

So far, IGF-I serum levels, determined by RIA, have been widely used in screening for GH deficieny or acromegaly. However, several limitations are obvious:

1. The normal range of IGF-I is low in young children making discrimination of subnormal levels difficult at that age.
2. A considerable number of children of small stature have, despite normal GH secretion, IGF-I levels in the subnormal
range. Therefore, the specificity and consequently the accuracy of the test for diagnosis of GH deficiency is limited.

The major advantages of IGFBP-3 over IGF-I are:

1. No extraction step is required prior to measurement thus improving test accuracy by simplifying the assay procedure.
2. The normal range in young children is comparatively high making the detection of subnormal levels more reliable.
3. Patients with GH deficiency have subnormal IGFBP-3 levels. In contrast, most of the small statured children with normal GH secretion have levels within the normal range (Figure 1).

The separation of these two groups is easy. A single measurement of the IGFBP-3 concentration is sufficient for the diagnosis of GH deficiency with high accuracy (7,18). In small statured children IGFBP-3 levels rise to normal range within several days of GH administration and remain normal during continuous GH treatment (Fig. 2). Therefore, serum IGFBP-3 measurements are also suited for evaluating the potential of a patient to respond to GH and for GH therapy monitoring (19).

In other patients of severe short stature, e.g. Ullrich-Turner syndrome or Silver-Russell syndrome, IGFBP-3 levels were found normal (8) reflecting normal GH secretion. In normal tall children and adolescents without excessive GH secretion or in patients with Sotos syndrome, IGFBP-3 levels are normal or slightly increased. In contrast, children with pituitary gigantism or adults with acromegaly have clearly elevated levels (Figure 3) (6,15) that normalize on successful treatment. Therefore, IGFBP-3 is also a useful parameter for the detection of excessive GH secretion and monitoring therapy efficacy. In precocious puberty, IGFBP-3 levels are clearly increased by chronological age, whereas patients with premature thelarche have IGFBP-3 levels in the upper normal range (15).
Figure 1: Serum IGFBP-3 levels in patients with short stature without GH deficiency (SS: constitutional delay of growth and adolescence, familial short stature, intra-uterine growth retardation) and in idiopathic or organic GH deficiency (GHD). The normal range is given by the 5th, 50th and 95th percentile.

Figure 2: IGFBP-3 levels in GH deficient children before and during GH treatment. Because of the age-dependence, values are given as the mean of standard deviation scores (SDS).
Figure 3: Serum IGFBP-3 levels in acromegaly. The normal range is given by the 5th, 50th and 95th percentile.

INTENDED USE
This radioimmunoassay kit is suited for measuring IGFBP-3 in human serum, plasma, or other human biological fluids (e.g. follicular fluid, seminal plasma) for diagnostic and scientific purposes. Its diagnostic value for GH deficiency screening is based on the high sensitivity and specificity of serum IGFBP-3 as a test for this diagnosis. States of GH excess may also be detected since IGFBP-3 levels are increased in that case. Due to its constant circadian concentration IGFBP-3 determination in a single blood sample may be sufficient as a screening test for these pathological situations prior to subjecting patients to further testing of GH secretion. IGFBP-3 determinations may also be suited for monitoring the efficacy of treatment and the patient’s compliance in GH deficiency and acromegaly.
PRECAUTIONS

General

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 is subject to the regulations of the national nuclear regulatory authorities.
Reagents with different lot numbers should not be mixed.

Reagents contain Sodium-Azide as preservative, however, highly diluted (0.02%). Sodium-Azide is very toxic, R-Phrases: 28, 32, 50/53 and S-Phrases 28, 45, 60, 61 must be considered.

First aid procedures:

Scin contact: Wash affected area thoroughly with water at least 15 minutes. Discard contaminated cloths and shoes. See a physician.

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. See a physician.

Ingestion: If swallowed, wash out mouth thoroughly with water, provided that the person is conscious. Immediately see a physician.

The handling of radioactive and potentially infectious material must comply with the following guidelines:
The material should be stored and used in a special designated area.
Do not eat, drink or smoke in these areas.
Never pipette the materials with the mouth.
Avoid direct contact with these materials by wearing laboratory coats and disposable gloves.
Spilled material must be wiped off immediately. Clean contaminated areas and equipment with a suitable detergent.
Unused radioactive material and radioactive waste should be disposed according to the recommendations of the national regulatory authorities.

Radioactivity
Before ordering or using radioactive materials, it is necessary to take the appropriate actions to ensure compliance with national regulations governing their use. Local rules in each establishment, which define actions and behaviour in the radioactivity working areas, should also be adhered to. The advice given here does not replace any local rules, instructions or training in the establishment, or advice from the radiation protection advisers. It is important to follow the code of good laboratory practice in addition to the specific precautions relating to the radionuclide I-125 used.

Iodine-125 has a radioactive half-life $T_{1/2}$ of 60 days and emits 35.5 keV gamma radiation, $27 - 32$ keV x-rays and no beta radiation. Shielding is effective done by lead, first half value layer is 0.02 mm lead, reduction to 10% is made by 0.2 mm.

To reduce the radiation dose time spent handling radioactivity should be minimized (plan ahead), and distance from source of radiation should be maximized (doubling the distance from the source quarters the radiation dose).

Formation of aerosols, e.g. by improper opening and mixing of vials or pipetting of solutions which may cause minute droplets of radioactivity become airborne, is a hazard and should be avoided.

Solutions containing iodine should not be made acidic, because this might lead to the formation of volatile elemental iodine.

As some iodo-compounds can penetrate rubber gloves, it is advisable to wear two pairs, or polyethylene gloves over rubber.
For cleaning of contaminated areas or equipment, the Iodine-125 should be rendered chemically stable by using alkaline sodium thiosulphate solution together with paper or cellulose tissue.

**METHODOLOGY**

**Assay Characteristics and Validation**

The radioimmunoassay for IGFBP-3 utilizes a specific high affinity polyclonal antibody for this protein. It recognizes quantitatively the complete IGFBP-3 complex and is unaffected by excess of IGF-I or IGF-II. Related molecules such as IGFBP-1 or IGFBP-2 show no cross-reaction in the assay (Figure 4), and the antibody is specific for primate IGFBP-3. The sensitivity of the assay is 0.5 ng/ml. Half-maximal displacement occurs at 11 ng/ml. The inter-assay variation has been found to be less than 9.0%, the intra-assay variation did not exceed 7.5%.

![Figure 4: Representative displacement curves](image)
The tracer is prepared by direct radioiodination of pure IGFBP-3 and standards refer to a stable derivative of IGFBP-3 having a molecular weight of 30.5 kDa determined by SDS-PAGE. The high sensitivity of the assay allows measurement of IGFBP-3 in small sample volumes which is limited by pipetting accuracy rather than the amount of IGFBP-3. Serum or plasma samples must be considerably diluted before measurement. No extraction step is required as with conventional IGF-I or IGF-II determinations.

Clinical Validation
Clinical validation was achieved by determination of IGFBP-3 levels in a large number of normal children and adults, normal short stunted children without GH deficiency, girls with Ullrich-Turner Syndrome, children with Silver-Russell Syndrome, patients with GH deficiency, children with familial tall stature, Sotos-Syndrome, patients with acromegaly, children with premature thelarche and precocious puberty (Tab. 1, Figures 1, 2, 3, 5 and 6).

Sample Handling and Storage
Serum and EDTA plasma levels are comparable regarding the IGFBP-3 quantification. Blood samples may be taken at any time of the day. Whole blood should be processed within two hours. Once separated the samples should be stored frozen at -20°C until measurement. IGFBP-3 levels are only modestly influenced by improper handling or storage and remain stable over several days at elevated temperatures in normal and in many clinical situations if undiluted. If diluted, however, stability is extremely decreased (see chapter Sample Preparation for necessary precautions). Store undiluted samples frozen in a
plastic vial. Avoid repeated freezing and thawing cycles, although in normal serum IGFBP-3 levels remained unchanged even after 10 cycles. Frozen samples remain stable over years. **Specimen Requirements**: 10 µl of serum or EDTA plasma.

**MATERIALS**

**Materials Provided**
The reagents and coated tubes listed below are sufficient for 100 determinations including the standard curve.

**2xDB)** Dilution Buffer, concentrate
(1 bottle, 125ml, 2-fold concentrated, blue coloured)

**R )** Capture Antibody: anti-rabbit-IgG, biotin-conjugated
(1 bottle, 5.5 ml, lyophilized)

**S )** Specific Antibody (rabbit-anti-hIGFBP-3)
(1 bottle, 5.5 ml, lyophilized, blue coloured)

**C )** Tracer (125I-IGFBP-3) (< 1.30 µCi or < 50 kBq)
(1 bottle, 11 ml, lyophilized, red coloured)

**F - J )** Standards. Concentrations (ng/ml) are given on vial-labels
(5 vials, 500 µl each, lyophilized)

**N)*** Control (human serum). Concentration (mg/l) is given on vial label
(1 vial, 100 µl, lyophilized)

**T)*** Tubes
(100 tubes, coated with streptavidin)
**Required Materials Not Provided**

1) Pipettes: 10 ml, 500 µl, 250 µl, 100 µl, 10 µl
   - 50 µl, 100 µl, 500 µl, and 1 ml repeating pipettes are recommended
2) Shaking device
3) Device for aspiration of liquids (e.g. connected to a water pump).
4) Gammacounter

**Reagent Storage and Preparation**

Store the kit after receipt at 2 - 8 °C until its expiry date. After reconstitution store the lyophilized reagents at –20 °C. Avoid repeated thawing and freezing cycles. The shelf-life of the components after opening is not affected, if used appropriately.

**2xDB)** Fill up to 250 ml with distilled water
   (∆→**Assay Buffer DB**)

**R)** Reconstitute with 5.5 ml reagent **DB (Assay Buffer)**
**S)** Reconstitute with 5.5 ml reagent **DB (Assay Buffer)**
**F-J)** Reconstitute each vial with 500 µl reagent **DB (Assay Buffer)**
**C)** Reconstitute with 11 ml reagent **DB (Assay Buffer)**
**N)** Reconstitute with 100 µl reagent **DB (Assay Buffer)**

Dilute the control according to sample dilution (e.g. 1:300)

Ensure that lyophilized materials are completely dissolved on reconstitution. It is recommended to keep reconstituted reagents at room temperature for half an hour and then to mix them vigorously with a Vortex mixer. This is important in particular for control **N**!
In the concentrated Dilution Buffer (2xDB) salt precipitates might develop due to cold storage - please ensure the complete solution of all components by warming up to room temperature.

Sample Preparation
Serum or plasma samples should be diluted 1:100 - 1:400-fold with Assay Buffer DB prior to measurement depending on the expected values. Usually a dilution of 1:300 is appropriate.

Important: Because IGFBP-3 is not stable in diluted solutions, please use only, chilled, preferably ice-cold Dilution Buffer DB. The time interval between the sample dilution and incubation should be as short as possible, i.e. the diluted samples should be processed fast as can.

Example: Mix 10 µl serum or plasma with 3 ml Assay Buffer DB (dilution factor: 1:301).

Simultaneous IGF-I determination:
By determination of the IGF-I concentration and the IGFBP-3 concentration from one sample, use one dilution of the sample for both determinations (provided that IGF-I is determined with the Mediagnost IGF-I RIA-Kit IGF-R22):

1) Dilute the samples according to the IGF-I working instruction with acidic Dilution Buffer DB (pH=2.1) 1:26 (e.g. 20 µl serum + 500 µl DB 2.1). This dilution is directly usable for the IGF-R22.

2) For IGFBP-3 determination transfer 90 µl neutral Dilution Buffer DB (pH=7.4) from the IGFBP-3 CT-RIA (IGF-R11) in the tubes. Subsequently add 10 µl of the 1:26 diluted sample from the IGF-I CT-RIA (IGF-R22, see step 1) to each tube. Dilution factor of the sample 1:260.
Dilution of control N should be carried out according to the dilution of serum or plasma samples.

**ASSAY PROCEDURE**

Samples (standards and patient specimen) should be determined in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

**Flow Chart of Assay Protocol**

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Tubes</th>
<th>DB F-N Samples</th>
<th>R</th>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>TC</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>100</td>
</tr>
<tr>
<td>3,4</td>
<td>NSB</td>
<td>DB: 150</td>
<td>50</td>
<td>---</td>
<td>100</td>
</tr>
<tr>
<td>5,6</td>
<td>Bo</td>
<td>DB: 100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>7-16</td>
<td>Standards</td>
<td>F-J: 100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>17,18</td>
<td>Control</td>
<td>N: 100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>19,20</td>
<td>Sample 1</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>21,22</td>
<td>Sample 2</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All volumes are given in µl.

1) Labelling of assay tubes should be done in the following order:
   1, 2 total counts (TC)
   3, 4 non-specific binding (NSB)
   5, 6 **Assay Buffer DB** (zero standard, B0)
   7 - 16 duplicates of **standards F to J**
   17,18 control N
   from 19 duplicates of **samples**.

2) Add 150 µl of reagent **DB** (**Assay Buffer**) to tubes 3 and 4.
3) Add **100 µl** of reagent **DB (Assay Buffer)** as zero standard, **B₀** to tubes 5 and 6

4) Add **100 µl** of reagents **F - J (standards)** to tubes 7-16
   - **7, 8** standard **F**
   - **9, 10** standard **G**
   - **11, 12** standard **H**; etc.

5) Add **100 µl** of diluted reagent **N (control)** to tubes 17 and 18.

6) Add **100 µl** of diluted sample to tubes 19 and 20, etc.

7) Add **50 µl** reagent **R (capture antibody)** beginning with tube 3.

8) Add **50 µl** reagent **S (specific antibody)** beginning with tube 5.
   - All solutions are coloured **blue**!

9) Add **100 µl** reagent **C (tracer)** to all tubes.
   - Mark tubes 1 and 2 (total counts), seal with a stopper or remove until step 13.
   - All solutions are coloured **violet**!

10) Incubation conditions:
   - **overnight** (at least 15 hours) at **room temperature** on a shaking device at **350 rpm**. Without shaking device, the tubes must be mixed thoroughly by a vortex mixer. Then incubate also overnight at room temperature (with slightly reduced binding), or, for **2 days** (or the weekend) at **2 - 8°C**.

11) Aspirate the liquid (except tubes 1 and 2 !) completely.
   - Take care that the coating of the tubes remains intact.
   - Depending on laboratory equipment and common laboratory practice, aspiration of the liquid can be replaced by total decantation.
12) Add 500 µl of reagent DB (Assay Buffer) to the tubes (except tubes 1 and 2 !).

13) Aspirate the liquid (see step 11).

14) Count the radioactivity of all tubes.

EVALUATION OF RESULTS

Establishing the Standard Curve:

The standards provided contain the following concentrations of IGFBP-3:

<table>
<thead>
<tr>
<th>Standard</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>27</td>
<td>81</td>
</tr>
</tbody>
</table>

1. Calculate the average counts (AC) of each pair of tubes. This gives the values for B.

2. Subtract the average counts (AC) of tubes 3 and 4 (non-specific binding NSB) from the mean counts of the standards, controls and patient samples. This gives the corrected values for B.

3. The corrected value from tubes 5 and 6 (Assay Buffer, DB) is $B_0$.

4. Calculate the percent bound ($\% B/B_0$):
   \[ \% B/B_0 = B/B_0 \times 100\% \]

5. Plot $\% B/B_0$ versus the standard concentrations on either semi-logarithmic or logit-log paper. For convenience, it is recommended to use computer assisted data reduction programs.

6. Quality control 1, calculate the non-specific binding NSB in
   \[ \% NSB/TC = NSB / \text{Total Counts TC} \times 100\% = (AC \text{ tubes 3 + 4} / AC \text{ tubes 1 + 2}) \times 100\%. \]
It should be < 8% (%NSB/TC < 8).

Quality control 2, calculate the percent bound of zero standard B₀: \( \frac{\%B_0/TC}{B_0/\text{Total Counts TC} \times 100\%} = \frac{\text{(AC tubes 5 + 6 - AC tubes 3 + 4)}}{\text{AC tubes 1 + 2} \times 100\%} \)

It should be > 25% (%B₀/TC > 25)

**EVALUATION OF SAMPLE CONCENTRATIONS:**
Read the concentration value (abscissa) corresponding to the % B/B₀ of the sample as in the example given below:

- average counts of NSB: 328 cpm
- average counts of zero standard (B₀): 5494 cpm
- average counts of sample: 2472 cpm

\[ \%B / B_0 = \frac{\text{cpm sample - NSB}}{\text{cpm B₀ - NSB}} \times 100\% \]
\[ = \frac{2472 - 328}{5494 - 328} \times 100\% \]
\[ = 41.5\% \]

For a 41.5% value on the y-axis (ordinate) a value of 16.15 ng/ml on the x-axis (abscissa) was obtained. Multiply the concentration value determined graphically or by aid of a computer program with the dilution factor (e.g. 301).

**Example:** 16.15 ng/ml x 301 = 4861 ng/ml = 4.86 mg/l.

**Concentration of control:**
The IGFBP-3 concentration of Control N should be within the following range (mean value ± 2 SD):

1.35 ± 0.34 mg/l
EXPECTED VALUES
IGFBP-3 levels are strongly age-dependent in children, less so in adults. The normal ranges in various age-groups which were log-normally distributed are given in Table 1 by the percentiles. A graphic presentation is shown in Figures 5 and 6. It is recommended for each laboratory to establish its own normal range.

LIMITATIONS
IGFBP-3 levels are strongly dependent on GH secretion. However, a number of factors influence its plasma concentration and should be taken into account for appropriate interpretation. Plasma levels decrease during fasting (more than 1 day), in malnutrition, malabsorption, cachexia, impaired hepatic function, hypothyroidism, and diabetes mellitus. They may also be decreased in chronic inflammatory disease and malignancy. Levels are increased in states of impaired renal function and precocious puberty. In clinical situations with hyperprolactinemia or in patients with craniopharyngeoma, normal levels may be observed despite GH deficiency. In certain physiological (e.g. pregnancy) and pathological states, IGFBP-3 may be degraded to smaller molecular size compounds (16,17) by specific proteases which affect IGFBP patterns seen in Western ligand blotting but have little influence on the outcome of RIA determinations.
Tab. 1: Serum levels of IGFBP-3 in healthy subjects at various ages. Individuals between 7 and 17 years of age were classified according to gender, as the pubertal peak occurs almost 2 years earlier in girls than in boys.

<table>
<thead>
<tr>
<th>Age group</th>
<th>0.1</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>95</th>
<th>99</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 week</td>
<td>0.25</td>
<td>0.33</td>
<td>0.42</td>
<td>0.48</td>
<td>0.57</td>
<td>0.64</td>
<td>0.77</td>
<td>0.93</td>
<td>1.05</td>
<td>1.23</td>
<td>1.41</td>
<td>1.81</td>
</tr>
<tr>
<td>1-4 weeks</td>
<td>0.49</td>
<td>0.62</td>
<td>0.77</td>
<td>0.86</td>
<td>0.99</td>
<td>1.10</td>
<td>1.29</td>
<td>1.52</td>
<td>1.68</td>
<td>1.93</td>
<td>2.16</td>
<td>2.68</td>
</tr>
<tr>
<td>1-3 months</td>
<td>0.55</td>
<td>0.70</td>
<td>0.87</td>
<td>0.98</td>
<td>1.13</td>
<td>1.25</td>
<td>1.48</td>
<td>1.75</td>
<td>1.94</td>
<td>2.23</td>
<td>2.52</td>
<td>3.14</td>
</tr>
<tr>
<td>3-6 months</td>
<td>0.64</td>
<td>0.80</td>
<td>0.98</td>
<td>1.10</td>
<td>1.25</td>
<td>1.38</td>
<td>1.61</td>
<td>1.88</td>
<td>2.07</td>
<td>2.37</td>
<td>2.65</td>
<td>3.24</td>
</tr>
<tr>
<td>6-12 months</td>
<td>0.71</td>
<td>0.88</td>
<td>1.07</td>
<td>1.19</td>
<td>1.25</td>
<td>1.35</td>
<td>1.48</td>
<td>1.72</td>
<td>2.00</td>
<td>2.19</td>
<td>2.49</td>
<td>2.76</td>
</tr>
<tr>
<td>1-3 years</td>
<td>1.02</td>
<td>1.21</td>
<td>1.41</td>
<td>1.53</td>
<td>1.69</td>
<td>1.82</td>
<td>2.05</td>
<td>2.31</td>
<td>2.48</td>
<td>2.74</td>
<td>2.98</td>
<td>3.47</td>
</tr>
<tr>
<td>3-5 years</td>
<td>1.08</td>
<td>1.30</td>
<td>1.52</td>
<td>1.66</td>
<td>1.84</td>
<td>1.99</td>
<td>2.25</td>
<td>2.55</td>
<td>2.75</td>
<td>3.05</td>
<td>3.33</td>
<td>3.91</td>
</tr>
<tr>
<td>5-7 years</td>
<td>1.19</td>
<td>1.42</td>
<td>1.66</td>
<td>1.81</td>
<td>2.01</td>
<td>2.16</td>
<td>2.44</td>
<td>2.76</td>
<td>2.97</td>
<td>3.29</td>
<td>3.59</td>
<td>4.2</td>
</tr>
<tr>
<td>7-9 years</td>
<td>1.25</td>
<td>1.48</td>
<td>1.73</td>
<td>1.88</td>
<td>2.07</td>
<td>2.22</td>
<td>2.50</td>
<td>2.81</td>
<td>3.02</td>
<td>3.33</td>
<td>3.61</td>
<td>4.22</td>
</tr>
<tr>
<td>boys</td>
<td>1.36</td>
<td>1.61</td>
<td>1.88</td>
<td>2.04</td>
<td>2.25</td>
<td>2.42</td>
<td>2.72</td>
<td>3.06</td>
<td>3.28</td>
<td>3.62</td>
<td>3.94</td>
<td>4.58</td>
</tr>
<tr>
<td>girls</td>
<td>1.47</td>
<td>1.73</td>
<td>1.99</td>
<td>2.15</td>
<td>2.36</td>
<td>2.52</td>
<td>2.81</td>
<td>3.14</td>
<td>3.35</td>
<td>3.67</td>
<td>3.97</td>
<td>4.57</td>
</tr>
<tr>
<td>9-11 years</td>
<td>1.56</td>
<td>1.90</td>
<td>2.20</td>
<td>2.38</td>
<td>2.62</td>
<td>2.80</td>
<td>3.13</td>
<td>3.50</td>
<td>3.75</td>
<td>4.11</td>
<td>4.45</td>
<td>5.16</td>
</tr>
<tr>
<td>boys</td>
<td>1.58</td>
<td>1.88</td>
<td>2.19</td>
<td>2.38</td>
<td>2.63</td>
<td>2.82</td>
<td>3.18</td>
<td>3.58</td>
<td>3.84</td>
<td>4.25</td>
<td>4.62</td>
<td>5.39</td>
</tr>
<tr>
<td>girls</td>
<td>1.62</td>
<td>1.90</td>
<td>2.24</td>
<td>2.46</td>
<td>2.74</td>
<td>2.97</td>
<td>3.38</td>
<td>3.85</td>
<td>4.17</td>
<td>4.65</td>
<td>5.10</td>
<td>6.02</td>
</tr>
<tr>
<td>11-13 years</td>
<td>1.62</td>
<td>1.89</td>
<td>2.24</td>
<td>2.46</td>
<td>2.76</td>
<td>2.99</td>
<td>3.42</td>
<td>3.91</td>
<td>4.24</td>
<td>4.75</td>
<td>5.22</td>
<td>6.20</td>
</tr>
<tr>
<td>boys</td>
<td>1.69</td>
<td>2.03</td>
<td>2.39</td>
<td>2.61</td>
<td>2.91</td>
<td>3.14</td>
<td>3.56</td>
<td>4.04</td>
<td>4.36</td>
<td>4.85</td>
<td>5.30</td>
<td>6.24</td>
</tr>
<tr>
<td>girls</td>
<td>1.70</td>
<td>2.02</td>
<td>2.36</td>
<td>2.57</td>
<td>2.84</td>
<td>3.05</td>
<td>3.44</td>
<td>3.88</td>
<td>4.17</td>
<td>4.61</td>
<td>5.01</td>
<td>5.86</td>
</tr>
<tr>
<td>13-15 years</td>
<td>1.62</td>
<td>1.93</td>
<td>2.26</td>
<td>2.46</td>
<td>2.73</td>
<td>2.93</td>
<td>3.31</td>
<td>3.74</td>
<td>4.02</td>
<td>4.45</td>
<td>4.85</td>
<td>5.67</td>
</tr>
<tr>
<td>boys</td>
<td>1.58</td>
<td>1.90</td>
<td>2.24</td>
<td>2.45</td>
<td>2.72</td>
<td>2.94</td>
<td>3.33</td>
<td>3.78</td>
<td>4.07</td>
<td>4.53</td>
<td>4.95</td>
<td>5.83</td>
</tr>
<tr>
<td>girls</td>
<td>1.55</td>
<td>1.86</td>
<td>2.20</td>
<td>2.41</td>
<td>2.68</td>
<td>2.90</td>
<td>3.29</td>
<td>3.74</td>
<td>4.04</td>
<td>4.50</td>
<td>4.92</td>
<td>5.80</td>
</tr>
<tr>
<td>15-17 years</td>
<td>1.44</td>
<td>1.75</td>
<td>2.08</td>
<td>2.29</td>
<td>2.56</td>
<td>2.78</td>
<td>3.18</td>
<td>3.64</td>
<td>3.95</td>
<td>4.42</td>
<td>4.86</td>
<td>5.78</td>
</tr>
<tr>
<td>boys</td>
<td>1.38</td>
<td>1.68</td>
<td>2.01</td>
<td>2.21</td>
<td>2.48</td>
<td>2.69</td>
<td>3.08</td>
<td>3.53</td>
<td>3.83</td>
<td>4.29</td>
<td>4.72</td>
<td>5.63</td>
</tr>
<tr>
<td>girls</td>
<td>1.34</td>
<td>1.64</td>
<td>1.96</td>
<td>2.16</td>
<td>2.42</td>
<td>2.63</td>
<td>3.02</td>
<td>3.46</td>
<td>3.76</td>
<td>4.22</td>
<td>4.65</td>
<td>5.55</td>
</tr>
<tr>
<td>17-20 years</td>
<td>1.28</td>
<td>1.58</td>
<td>1.90</td>
<td>2.10</td>
<td>2.37</td>
<td>2.58</td>
<td>2.98</td>
<td>3.44</td>
<td>3.75</td>
<td>4.23</td>
<td>4.67</td>
<td>5.62</td>
</tr>
</tbody>
</table>

Serum levels are given as mg/ml, determined with IGFBP-3 RIA (Blum et al. 1990)
REFERENCES


### SUMMARY OF THE ASSAY

<table>
<thead>
<tr>
<th>Reagent preparation</th>
<th>Reconstitution</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution Buffer (2xDB)</td>
<td></td>
<td>Before use 1:2 with A.dest.</td>
</tr>
<tr>
<td>Capture Antibody (R) in 5.5 ml Sample Buffer (DB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific Antibody (S) in 5.5 ml Sample Buffer (DB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracer : $^{125}$I-IGFBP-3 In 11 ml Sample Buffer (DB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standards (F-J) in 500 µl Sample Buffer (DB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (N) in 100 µl Sample Buffer (DB) 1:300 with DB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dilute Sample with cold (2-8°C) Sample Buffer (DB) e.g. 1:300 +process fast as can

### Assay Procedure in Double Determination

<table>
<thead>
<tr>
<th>Nr. of tube</th>
<th>Contents of Tubes</th>
<th>DB F-N Samples</th>
<th>R</th>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>Total Counts</td>
<td></td>
<td>–</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>3,4</td>
<td>NSB</td>
<td>150 DB</td>
<td>50</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>5,6</td>
<td>B₀</td>
<td>100 DB</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>7-16</td>
<td>Standards</td>
<td>100 F-J</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>17,18</td>
<td>Control</td>
<td>100 N</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>19,20</td>
<td>Sample 1</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>21,22 (etc)</td>
<td>Sample 2 (etc)</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

**After addition**
- coloration deeper blue
- coloration violet

**Addition of Reagent [µl]**

<table>
<thead>
<tr>
<th>Nr.:1,2 remove until counting the activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix other tubes with a Vortex-Mixer</td>
</tr>
</tbody>
</table>

**Incubation**, over night (at least 15 hours), at RT, 350 rpm

Aspirate the liquid completely. Take care that the coating of the tubes remains intact.

Add 500 µl of reagent DB (Assay Buffer) to the tubes

Aspirate the liquid completely (see above)

Count the radioactivity of all tubes.