INTENDED USE
Immunoenzymatic colorimetric assay for the quantitative determination of human Insulin-like Growth Factor 1 (IGF-1) concentration in human serum. IGF-1 ELISA Assay kit is intended for research use only and not for diagnostics or therapeutic purposes.

1. CLINICAL SIGNIFICANCE
Insulin-like growth factor 1 (IGF-1) or Somatomedin-C (SM-C) is a basic 70 amino acid single chain polypeptide (MW : 7649 Da) similar to proinsulin (50% sequence homology), and to the other well-characterized member of the somatomedin family : IGF II (67AA, 70 % sequence homology with IGF-1). IGF-1 is the most important factor, which mediates the growth promoting actions of growth hormone, a pituitary hormone with highly fluctuating blood levels due to pulsatile release. The blood concentration of IGF-1 is more stable due to the binding to carrier proteins. The concentration of the predominant binding protein (MW 53000) as well as the production of IGF-1, are regulated by growth hormone. IGF-1 is produced by the liver, and other tissues, and it has endocrine, paracrine and autocrine activities. It stimulates growth and regulates differentiation of various tissues, displays insulin-like activities and promotes cartilage growth. Although GH is the most important factor controlling IGF-1 secretion and concentration, other factors are also determinant: the age (with a peak at adolescence), the sex, the nutritional status, and other hormones (oestrogen, thyroxin, prolactin, ...). Specific trophic stimuli mainly control IGF-1 secretion in the local microenvironment of a particular organ (paracrine activities), while blood IGF-1 concentration is the most important variable for balanced systemic growth (endocrine activities).

Clinical applications
Growth retardation: Growth retardation may be due to several causes, among which deficient GH production (hypopituitarism), which is associated with low IGF-1 blood levels. Because of the difficulties to get interpretable results from GH measurements (by dynamic multiple or stimulation tests), the determination of the stable IGF-1 concentration in plasma is often considered as a simple screening test to evaluation “GH impregnation” of the patient before deciding more extensive investigations. In several clinical situations with impaired growth, low IGF-1 levels may be observed despite normal or high GH production (i.e. malnutrition, chronic diseases states, some genetic dwarfs like Pygmies, ...). Interestingly, children with discrete GH neuro-secretory dysfunction may display low IGF-1 values despite normal GH levels by conventional testing. The results of IGF-1 assay must be interpreted cautiously by considering the normal variations of IGF-1 during childhood and adolescence (see Rosenfeld et al).

Acromegaly: IGF-1 levels are elevated in acromegaly (excess production of GH) and may serve as an indicator of disease severity. Results are more readily interpreted because the normal values are more easily defined in adults. IGF-1 measurements are also useful to monitor treatment.

Research: IGF-1 kit is an invaluable tool to study the modifications of this growth factor during physiologic (i.e. pregnancy) or pathologic (i.e. diabetes) situations, and the local regulation of IGF-1 production in relation to its paracrine and autocrine activities (wound healing, organ regeneration, neoplastic growth, foetal development, gonadal regulation, etc).

2. PRINCIPLE OF THE METHOD
IGF-1 ELISA Assay kit is a solid phase immunoenzymatic assay performed on polystyrene microtiterplates. In the present kit, DiaMetra has introduced a pre-treatment step in order to improve the clinical performance of the assay. It is well established that the binding proteins interfere with the assay for IGF-1. The pre-treatment step used by Diametra is the acid-alcohol procedure of Daughaday et al. (8).
A fixed amount of IGF-1-labelled with horseradish peroxidase (HRP), compete with unlabelled IGF-1 present in the calibrators, controls and samples for a limited number of binding sites on a specific antibody. After 1 hour incubation at room temperature, the microtiterplate is washed to st...
and incubated for 15 min. The reaction is stopped with the addition of Stop Solution and the microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the IGF-1 concentration. A calibration curve is plotted and IGF-1 concentration in samples is determined by interpolation from the calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (6 vials, lyophilized)
   - CAL0
   - CAL1
   - CAL2
   - CAL3
   - CAL4
   - CAL5
   Controls concentration is lot-specific and is indicated on the Certificate of Analysis

3. Conjugate (1 vial, 0.2 mL, 101X concentrate)
   - IGF-1 conjugated with Horseradish peroxidase (HRP)

4. Conjugate buffer (1 vial, 11.5 mL)
   - Phosphate buffer with preservatives

5. Coated Microplate (1 breakable microplate)
   - Anti IGF-1 antibodies coated on the microplate

6. TMB Substrate (1 vial, 25 mL)
   - H₂O₂-Tetramethylbenzidine (avoid any skin contact)

7. Stop Solution (1 vial, 12 mL)
   - Hydrochloridic acid 1N (avoid any skin contact)

8. 200X Conc. Wash Solution (1 vial, 10 mL)
   - Tris HCl buffer

9. Pre-treatment solution (1 vial, 20 mL)
   - Buffer solution with ethanol

10. Reconstitution solution (1 vial, 10 mL)
    - Buffer solution with ethanol

11. Neutralization solution (1 vial, 30 mL)
    - Phosphate buffer with sodium azide

3.2. Reagents necessary not supplied

- Distilled water.

3.3. Auxiliary materials and instrumentation

- Automatic dispenser.
- Microplates reader (450 nm, 630-650 nm)
- Polypropylene tubes for neutralization step (optional: microcentrifuge tubes)

- Microcentrifuge or Centrifuge

4. WARINGS

- This IGF-1 ELISA Assay kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Thymol or Sodium Azide (NaN₃) as preservative. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted hydrochloric acid solution. Hydrochloric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all IGF-1 ELISA Assay kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
• Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any IGF-1 ELISA Assay component beyond their expiry date.
• If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
• The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
• It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
• Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
• Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
• Maximum precision is required for reconstitution and dispensation of the reagents.
• Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the IGF-1 ELISA Assay.
• Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of Calibrators (C₀…C₅)
Reconstitute the Calibrators with 1.0 mL of Reconstitution solution.
Calibrators concentration is indicated on the labels. After reconstitution, the Calibrators are stable 1 week at 2-8°C. For longer period, aliquot the Calibrators and store at -20°C for maximum 3 months; avoid successive freezing and thawing.

1 ng of the calibrator preparation is equivalent to 1 ng of the NIBSC 1st WHO IS 02/254.

6.2. Preparation of Controls
Reconstitute the Controls with 1.0 mL of distilled water.
Controls concentration is indicated on the Certificate of Analysis. After reconstitution, the Controls are stable 1 week at 2-8°C. For longer period, aliquot the Controls and store at -20°C for maximum 3 months; avoid successive freezing and thawing.

6.3. Preparation of Conjugate
Just before the assay, dilute the Conjugate 101X concentrate (reagent 3) with Conjugate buffer (reagent 4); for example, dilute 20 μL of 101X Conjugate in 2 mL of Conjugate buffer. Use a vortex to homogenize. The diluted Conjugate is stable for 4 hours at room temperature (22-28°C).

Avoid exposure of concentrated and diluted Conjugate to direct sunlight.

6.4. Preparation of the Sample
The determination of IGF-1 with this kit must be performed in human serum. The serum should be stored at 2-8°C or at -20°C if the determination is not performed on the same day of the sample connection. Avoid repetitive freezing and thawing of samples. Prior to use all samples should be at room temperature (22-28°C); it is recommended to vortex the samples before using. If you need to dilute the samples, use the Calibrator 0.

6.5. Preparation of Wash Solution
Before assaying, dilute the amount needed of the "200X Conc. Wash Solution" with distilled water; for example, dilute 1 mL of concentrated wash solution with 199 mL of distilled water. Mix well before using. Discard unused diluted wash solution at the end of the day. Once opened, the "200X Conc. Wash Solution" is stable at 2-8°C until the expiry date.

6.6. Procedure
• Allow all reagents to reach room temperature (22-28°C). At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
• Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
• To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
• As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C₀-C₅), two for each Control and two for each sample.

Before assaying into the microplate, you need to pre-treat each sample and control (not the calibrators) according to one of the following procedures.

6.6.1. Pre-treatment step with a microcentrifuge tube
1. Label one microcentrifuge tube (for extraction) and one polypropylene tube (for neutralization) for each sample and control.
2. Dispense 100 μL of each sample and control into the microfuge tube.
3. Add 400 μL of "Pre-treatment solution" into the tube.
4. Close the tube, vortex and incubate 30 minutes at room temperature (22-28°C).
5. Centrifuge for 2 minutes at 10000 rpm.
6. Take 100 μL of the supernatant and transfer it into the polypropylene labelled tube.
7. Add 600 μL of the "Neutralization solution" to the tube.
8. Vortex the tube to mix, and assay the sample according to the assay procedure.

6.6.2. Pre-treatment step with a polypropylene tube
If you don’t have a microcentrifuge tube and a microcentrifuge, you can proceed as follow.
1. Label two polypropylene tubes (one for extraction, the other one for neutralization) for each sample and control.
2. Dispense 100 μL of each sample and control into the extraction tube.
3. Add 400 μL of "Pre-treatment solution" into the tube.
4. Close the tube, vortex and incubate 30 minutes at room temperature (22-28°C).
5. Centrifuge for 15 minutes at ≥ 3000 rpm.
6. Take 100 μL of the supernatant and transfer it into the other polypropylene labelled tube.
7. Add 600 μL of the "Neutralization solution" to the tube.
8. Vortex the tube to mix, and assay the sample according to the assay procedure.

6.6.3. Assay procedure

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Calibrator</th>
<th>Pre-treated Sample/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator C₀⁻C₅</td>
<td>50 μL</td>
<td>50 μL</td>
</tr>
<tr>
<td>Pre-treated Sample/Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugate</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
</tbody>
</table>

Incubate 1 hour at room temperature (22-28°C) on a shaker at 500 rpm. Remove the contents from each well. Wash the wells 3 times with 400 μL of diluted wash solution.

**Important note:** During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>200 μL</th>
<th>200 μL</th>
</tr>
</thead>
</table>

Incubate 15 minutes in the dark at room temperature (22-28°C) on a shaker at 500 rpm.

<table>
<thead>
<tr>
<th>Stop Solution</th>
<th>100 μL</th>
<th>100 μL</th>
</tr>
</thead>
</table>

Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 630-650 nm within 5 minutes.

7. **RESULTS**
1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. Calculate for each calibrator, control and sample:

   \[
   B/B₀ (%) = \frac{\text{OD (Calibrator or Control or Sample)}}{\text{OD (Calibrator 0)}} \times 100
   \]

4. Using either linear-linear or semi-logarithmic graph paper, plot the B/B₀ (%) values for each calibrator point as a function of the B/B₀ (%) values for each calibrator point. Reject obvious outliers.
5. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
6. By interpolation of the sample B/B₀ (%) values, determine the IGF-1 concentrations of the samples from the calibration curve

**Conversion factor:**

\[
\text{ng/mL} = \text{nmol/L} \times 0.13
\]

\[
\text{nmol/L} = \text{ng/mL} \times 7.65
\]

8. **QUALITY CONTROL**

Each laboratory should assay controls for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Controls containing sodium azide interfere with the assay, thus use of sodium azide must be avoided.

Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

9. **REFERENCE VALUES**

These values are given only for guidance.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>Median</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>30</td>
<td>37</td>
<td>14 - 154</td>
</tr>
<tr>
<td>6 - 8</td>
<td>20</td>
<td>93</td>
<td>45 - 213</td>
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<td>9 - 11</td>
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<td>53 - 453</td>
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<td>12 - 15</td>
<td>24</td>
<td>313</td>
<td>103 - 753</td>
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<tr>
<td>16 - 20</td>
<td>30</td>
<td>203</td>
<td>99 - 655</td>
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<td>21 - 25</td>
<td>9</td>
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<tr>
<td>40 - 54</td>
<td>35</td>
<td>156</td>
<td>69 - 343</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>25</td>
<td>117</td>
<td>33 - 232</td>
</tr>
</tbody>
</table>
### Females (ng/mL)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>Median</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>50</td>
<td>57</td>
<td>21 - 262</td>
</tr>
<tr>
<td>6 - 8</td>
<td>18</td>
<td>207</td>
<td>89 - 485</td>
</tr>
<tr>
<td>9 - 11</td>
<td>19</td>
<td>354</td>
<td>99 - 708</td>
</tr>
<tr>
<td>12 - 15</td>
<td>31</td>
<td>274</td>
<td>100 - 744</td>
</tr>
<tr>
<td>16 - 20</td>
<td>29</td>
<td>248</td>
<td>73 - 522</td>
</tr>
<tr>
<td>21 - 25</td>
<td>9</td>
<td>171</td>
<td>83 - 511</td>
</tr>
<tr>
<td>26 - 39</td>
<td>20</td>
<td>165</td>
<td>101 - 267</td>
</tr>
<tr>
<td>40 - 54</td>
<td>17</td>
<td>121</td>
<td>60 - 271</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>14</td>
<td>109</td>
<td>69 - 189</td>
</tr>
</tbody>
</table>

*The ranges are expressed as 2.5% to 97.5% percentiles.

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

### 10. PERFORMANCE AND CHARACTERISTICS

#### 10.1. Precision

**10.1.1. Intra Assay**

Within run variation was determined by replicate (10x) the measurement of two different sera in one assay. The within assay variability is ≤ 8.9%.

**10.1.2. Inter Assay**

Between run variation was determined by replicate (10x) the measurement of two different sera in different lots. The between assay variability is ≤ 12.9%.

#### 10.2. Specificity

The percentages of cross-reaction for the anti IGF-1 estimated by comparison of the concentration yielding a 50% inhibition are respectively:

- IGF-1: 100%
- IGF-2: 0.7%
- Insulin: ND
- GH: ND

#### 10.3. Accuracy

The recovery of 15-30-100-250-400-600 ng/mL of IGF-1 added to samples gave an average value (±SD) of 106.9% ± 9.5% with reference to the original concentrations. Dilution test was conducted by diluting two samples until 1:32 with Calibrator 0 after extraction; dilution test gave an average value (±SD) of 100.4% ± 9.0%.

#### 10.4. Detection limit

The lowest detectable concentration of IGF-1 that can be distinguished from the Calibrator 0 is 7.8 ng/mL.

### 11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

### BIBLIOGRAPHY


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DiaMetra S.r.l. Headquarter: Via Garibaldi, 18 – 20090 SEGRATE (MI) Italy
Tel. +39-02-2139184
Fax +39-02-2133354.

Manufactury: Via Pozzuolo 14, 06083 SPELLO (PG) Italy
Tel. +39-0742-24851
Fax +39-0742-316197
E-mail: info@diametra.com