**Human Intact Proinsulin ELISA**

Product Data Sheet

Cat. No.: RZ193094100

European Union: [IVD] [CE]

Rest of the world: For research use only!
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This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!
1. INTENDED USE

The RZ193094100 Human Intact Proinsulin ELISA is a sensitive two-site sandwich enzyme immunoassay for the quantitative measurement of human intact proinsulin.

2. STORAGE, EXPIRATION

Store the kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Proinsulin (PI), a polypeptide of 9390 MW (86 amino acids) is synthetized in the β cells of the Islets of Langerhans in the pancreas. The sequence of proinsulin is highly conserved in mammalian species and is homologous with IGF-1 and IGF-2. This protein is processed to C-peptide and insulin forms. Both are secreted in equimolar amounts into the blood. In normal individuals, proinsulin is present in the circulation in very low concentrations (typical basal values 2-6 pmol/l). The level of proinsulin in serum can be a reflection of β cell status and a consequence of dysfunction of PI processing and/or secretion. Proinsulin like material is increased in clinical conditions as insulinoma, familial hyperinsulinemia, non-insulin dependent diabetes mellitus.

4. TEST PRINCIPLE

The microtiter plates are coated with a monoclonal antibody (S2) specific for epitope at the C-peptide/insuline A chain junction. S2 is able to bind intact PI, des (31,32)-PI and split (32,33)-PI but not insulin, C-peptide and the other “des” and “split” forms.

Prior to use a blocking buffer is added to the allocated wells. An aliquot of patient sample is then added to the wells and incubated. The wells are then washed to remove unbound antibody and other serum compounds. In a second incubation time, an enzyme labelled anti-proinsulin antibody (S53) is incubated in the wells. This antibody is specific for the epitopes at insulin β chain/C-peptide junction. S53 is able to bind to intact PI, des (64,65)-PI and split (65,66)-PI but not insulin, C-peptide and other “des” and “split” forms. The combination of the two Mabs has the ability to detect only the intact human proinsulin.
After washing away any unbound enzyme labelled anti-proinsulin antibody, the enzyme activity is measured by adding a substrate solution. The intensity of colour development is proportional to the concentration of proinsulin in the patient sample.

5. PRECAUTIONS

- **For in vitro diagnostic use only**
- Use disposable gloves while handling potentially infectious material and performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- All material of human origin used for the preparation of this kit is tested negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents used for the assay must be considered potentially infectious; therefore, the assay waste must be decontaminated and disposed off, in accordance with established safety procedures. Disposable ignitable material must be incinerated; disposable non-ignitable material must be sterilized in autoclave for at least 1 hour at 121°C. Liquid wastes must be added with sodium hypochlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base before treating with sodium hypochlorite.
- Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.
- Some reagents contain sodium azide as preservative; to prevent buildup of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.
- Chromogenic substrate and Stop solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident rinse thoroughly with running water.
- Calibrators contain Thimerosal. This product is highly toxic by inhalation, swallowing and contact with skin. Keep away from food and drink.
- Conjugate contains Proclin. This product is highly toxic by inhalation, swallowing and skin contact. Keep away from food and drink.
- Wear protective clotters and gloves. In case of contact with skin or eyes, rinse thoroughly with water. In case of accident, consult immediately a physician and show him/her the product label.
- Stop solution contains sulfuric acid (4.9% w/w) (H331/H314/H315)

Risk phrases

- H331 toxic by inhalation
- H314 causes burns
- H315 irritating skin
6. TECHNICAL HINTS

- Do not mix reagents from different lot numbers or from other manufacturers.
- Do not freeze kits.
- Strict adherence to the specific time and temperature of incubations is recommended for accurate results.
- Allowing the micro well strip and reagents to equilibrate to room temperature before opening and using them.
- Do not use reagents after their expiration dates.
- Incomplete of inefficient washing will cause poor precision and high background.
- Use thoroughly clean glassware, free from contamination of metal ions or oxidating substances.
- Use distilled water, stored in clean containers.
- Microbial contaminated serum or specimens containing heavy, visible particulate should not be used.
- Cross contaminations of reagents or sample could cause false results. Use a clear, fresh, disposable pipette tip for each reagent of specimen manipulation.
- Do not expose the substrate to light during storage or incubation.
- Follow exact incubation times. Dispense Chromogen and Stop solution in no more than 3-4 minutes; dispense the two reagents in the same sequence.
- Residual amounts of sodium azide (NaN3) can destroy the conjugate's enzymatic activity.
- Traces of hypochlorite of soda can destroy many reagents' biological activity.

A variety of factors influence the assay performances. These include the accuracy and reproducibility of pipetting technique, the photometer used, timing bias during the assay.

7. REAGENT PROVIDED

1. Antibody Coated Microtiter Strips: 96 breakable wells coated with a proinsulin specific antibody. Keep unused wells at 2-8°C, protected from moisture in the provided aluminium bag and carefully sealed.
   Store at 2 – 8°C

   Preservatives: Merthiolate.
   Store lyophilized at 2 – 8°C
3. **Calibrators 1-5**: 5 vials of lyophilised calibrator. *Reconstitute with 1 ml of distilled water.*  
Blue coded. After reconstitution, keep the calibrator at –20°C (freeze-defreeze cycle: max 2 times). For the exact value, refer to the CoA.  
**The calibrators are calibrated against the 1st International Standard WHO 09/296.**  
Preservatives: Merthiolate.  
Store lyophilized at 2 – 8°C

4. **Quality Control HIGH and LOW**: 2 vials of lyophilised sample. *Reconstitute with 1 ml of distilled water.* Blue coded. After reconstitution, keep the control serum at –20°C (freeze-defreeze cycle: max 2 times)  
For the exact value, refer to the CoA.  
Preservative: Merthiolate.  
Store lyophilised at 2 – 8°C.

5. **Conjugate Solution**: 1 vial (11 ml) of anti-human proinsulin conjugated to horseradish peroxidase (HRPO). Preservative: Proclin 300  
Store at 2 – 8°C.

6. **Blocking buffer**: 1 vial (1.5 ml) of murin IgG in phophate buffer. Ready for use.  
Preservative: Merthiolate.  
Store at 2 – 8°C.

7. **Wash Solution Conc. (10x)**: 1 vial (40 ml) of buffer with Tween 20. Bring the vial content to 400 ml (final volume) with distilled water. The diluted washing solution is stable for 6 months at 2 - 8°C. Preservatives: Sulfate Streptomycin and Amphotericin.  
Store at 2 – 8°C

8. **Substrate Solution**: 1 vial (25 ml) of Tetramethylbenzidine in citrate-phosphate buffer and DMSO. Ready for use.  
Store at 2 – 8°C

9. **Stop Solution**: 1 vial (15ml) of 0.5M H₂SO₄. Ready for use.  
Store at 2 – 8°C
8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Adjustable, automatic micropipettes with disposable tips.
- Graduated cylinder.
- Aspiration pump or automated well washing device.
- Microtiterplate spectrophotometer capable of measuring absorbances within a 0-3.0 A interval at 450 and 405 nm.
- Millimetric graph paper.
- Orbital shaker adjustable at 150 rpm.
- Distilled H₂O.

9. PREPARATION OF SAMPLES

Both serum and plasma may be used in the assay, although values with serum are slightly lower than plasma. Avoid using hemolized, lipemic or bacterially contaminated sera. Thoroughly mix thawed specimens before assay and avoid repeated freeze/thawing cycles which may cause loss of antibody activity and give erroneous results. Samples may be stored at 2°- 8°C for up to 24 hours. For long term storage, samples should be stored frozen at -20°C or lower.
10. ASSAY PROCEDURE

- Allow reagents and samples to warm up at room temperature.
- Mix samples by inversion before use.
- The samples are not diluted.

1) Allocate the wells of Microtiter Plate for calibrators, control sera and samples.
2) Pipette 10 µl of Blocking Buffer directly into the bottom of the wells.
3) Pipette 100 µl of each Calibrators, Quality Controls and sample into the corresponding wells.
4) Incubate for 30 minutes at room temperature on an orbital shaker (150rpm) (NB. The incubation time begins after the last sample addition.)
5) Wash the wells 3 times with 300 µl of diluted Washing solution. Aspirate all liquid from the wells.
6) Add 100 µl of Conjugate Solution into the wells.
7) Incubate for 60 minutes at room temperature on an orbital shaker (150rpm).
8) Wash the wells 3 times with 300 µl of diluted Washing solution. Aspirate all liquid from the wells.
9) Pipette 200 µl of Substrate Solution into each well.
10) Incubate 15 minutes at room temperature on an orbital shaker (150rpm), avoid direct light exposure (NB. The incubation time begins after the first TMB addition)
11) Pipette 100 µl of Stop Solution into each well.
12) Read the absorbance of the wells (450, 405 and 620nm (as reference)). Reading must be completed within 20 minutes from the end of the assay.

<table>
<thead>
<tr>
<th>Wells</th>
<th>Reagents</th>
<th>Blank</th>
<th>Calibrator (0-5)</th>
<th>Quality Controls</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocking buffer</td>
<td>–</td>
<td>–</td>
<td>10 µl</td>
<td>10 µl</td>
<td>10 µl</td>
</tr>
<tr>
<td>Calibrator (0-5)</td>
<td>–</td>
<td>–</td>
<td>100 µl</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Quality Controls</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100 µl</td>
<td>–</td>
</tr>
<tr>
<td>Samples</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100 µl</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Conjugate solution</td>
<td>–</td>
<td>–</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Substrate solution</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td></td>
</tr>
<tr>
<td>Stop solution</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td></td>
</tr>
</tbody>
</table>

- Incubate: 30’ at RT, orbital shaker (150 rpm)
- Aspirate and wash: 3 x 300 µl

- Incubate: 60’ at RT, orbital shaker (150 rpm)
- Aspirate and wash: 3 x 300 µl

- Incubate: 15’ at RT, orbital shaker (150 rpm)
- Read: 450-405 nm and 620 nm as reference
11. CALCULATIONS

Automatically: select semi-logarithmic Cubic spline graph on the microplate reader
Manually: draw a graph as follows:
1. ORDINATES: measured optical density value for each calibrator
2. ABSCISSA: decimal logarithm of concentrations of each calibrator
Plot optical density values of the samples on the curve
O.D. = f (Log concentration C)
Read abscissa concentration logarithm (X)
Calculate C (concentration): C=10 x

In order to obtain a better sensitivity, the present method employs spectrophotometric reading at two wavelengths (450 and 405 nm). For all O.D. overflow at 450nm, multiplied the O.D. 405nm by the correction factor calculated by the ratio between O.D. 450nm and O.D. 405nm. To establish this factor, used a sample reading at 405nm and 450nm.

Example of calculation:

The values shown below must be considered as an example and must not be used in place of experimental data.

<table>
<thead>
<tr>
<th>Description</th>
<th>Concentration (pmol/l)</th>
<th>O.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 0</td>
<td>0</td>
<td>0.040</td>
</tr>
<tr>
<td>Calibrator 1</td>
<td>3.6</td>
<td>0.255</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>11.1</td>
<td>0.746</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>20.4</td>
<td>1.391</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>47.5</td>
<td>3.116</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>99.5</td>
<td>6.129</td>
</tr>
<tr>
<td>Quality Control Low</td>
<td>14.1</td>
<td>0.961</td>
</tr>
<tr>
<td>Quality Control High</td>
<td>27.7</td>
<td>1.845</td>
</tr>
<tr>
<td>P1</td>
<td>8.7</td>
<td>0.581</td>
</tr>
<tr>
<td>P2</td>
<td>24.1</td>
<td>1.620</td>
</tr>
<tr>
<td>P3</td>
<td>30.1</td>
<td>1.992</td>
</tr>
<tr>
<td>P4</td>
<td>83.7</td>
<td>5.352</td>
</tr>
</tbody>
</table>
12. PERFORMANCE CHARACTERISTICS

- **Sensitivity**
  The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Calibrator (mean value + 2 S.D.). This dose is 0.6 pmol/l.

- **Specificity**
  Patient samples may contain human anti-mouse antibodies (HAMA), which are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. This assay has been designed to minimize interference from HAMA-containing specimens with the use of blocking HAMA interferences (Blocking Buffer). Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

- **Cross-reactivity**
  The following peptides were tested and no cross-reactivity has been observed:

  Human Insulin:   < 10 000 pmol/l
  Human C-Peptide: 50 000 pmol/l
  Des (31,32)-Proinsulin: < 200 pmol/l
  Split (32,33)-Proinsulin: 5 000 pmol/l
  Des (64,65)-Proinsulin: 200 pmol/l
  Split (65,66)-Proinsulin: 1 000 pmol/l
- **Precision**

**Intra-assay**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± S.D. (pmol/l)</th>
<th>Standard Deviation</th>
<th>CV (%)</th>
<th>Replicates No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9.4 ± 0.3</td>
<td>0.3</td>
<td>3.2</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>23.7 ± 0.7</td>
<td>0.7</td>
<td>3.0</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>41.9 ± 1.2</td>
<td>1.2</td>
<td>2.9</td>
<td>20</td>
</tr>
</tbody>
</table>

**Inter-assay**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± S.D. (pmol/l)</th>
<th>Standard Deviation</th>
<th>CV (%)</th>
<th>Replicates No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.9 ± 0.2</td>
<td>0.2</td>
<td>2.5</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>14.1 ± 0.2</td>
<td>0.2</td>
<td>1.4</td>
<td>9</td>
</tr>
<tr>
<td>C</td>
<td>29.1 ± 0.4</td>
<td>0.4</td>
<td>1.4</td>
<td>9</td>
</tr>
</tbody>
</table>

- **Spiking Recovery**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Measured (pmol/l)</th>
<th>Expected (pmol/l)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>8.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S1+Cal 0</td>
<td>4.5</td>
<td>4.3</td>
<td>104.7</td>
</tr>
<tr>
<td>S1+Cal 1</td>
<td>6.0</td>
<td>6.1</td>
<td>98.4</td>
</tr>
<tr>
<td>S1+Cal 2</td>
<td>10.1</td>
<td>9.9</td>
<td>102.0</td>
</tr>
<tr>
<td>S1+Cal 3</td>
<td>15.1</td>
<td>14.5</td>
<td>104.1</td>
</tr>
<tr>
<td>S1+Cal 4</td>
<td>28.7</td>
<td>28.1</td>
<td>102.1</td>
</tr>
<tr>
<td>S1+Cal 5</td>
<td>56.9</td>
<td>54.1</td>
<td>105.2</td>
</tr>
</tbody>
</table>

- **Dilution Linearity**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Observed (pmol/l)</th>
<th>Expected (pmol/l)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1</td>
<td>22.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1/2</td>
<td>11.8</td>
<td>11.2</td>
<td>105</td>
</tr>
<tr>
<td>1/4</td>
<td>6.4</td>
<td>5.6</td>
<td>114</td>
</tr>
<tr>
<td>1/6</td>
<td>4.0</td>
<td>3.7</td>
<td>107</td>
</tr>
<tr>
<td>1/8</td>
<td>2.8</td>
<td>2.8</td>
<td>100</td>
</tr>
<tr>
<td>1/10</td>
<td>2.0</td>
<td>2.2</td>
<td>90</td>
</tr>
</tbody>
</table>
- Normal Values

The normal values determined are only indicative since they may be affected by various agents. We recommend that each laboratory establish its own normal range.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of samples</th>
<th>Mean ± SD (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non obese fasting patients</td>
<td>16</td>
<td>2.56 ± 1.28</td>
</tr>
<tr>
<td>Fasting IDDM</td>
<td>9</td>
<td>4.67 ± 3.85</td>
</tr>
<tr>
<td>Non-fasting IDDM</td>
<td>13</td>
<td>16.25 ± 17.75</td>
</tr>
</tbody>
</table>

The regression analysis for a plasma/serum comparison equation is:

\[ Y \text{ (plasma value)} = 1.20 \times X \text{ (serum value)} + 1.17 \]

Correlation Coefficient \( r = 0.98 \) (n=48)
13. REFERENCES

References to Proinsulin:


For more references on this product see our WebPages at www.biovendor.com
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