DRG® Proinsulin ELISA (EIA-1560)

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IVD

1 INTRODUCTION

1.1 Intended Use

The **DRG Proinsulin ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Proinsulin (intact) in serum and plasma.

1.2 Summary and Explanation

The measurement of Proinsulin in serum can provide valuable information for the diagnosis of insulinomas. Proinsulin levels have also been shown to be elevated in non-insulin dependent diabetics (NIDDM), newly diagnosed insulin dependent diabetics (IDDM) and other clinical situations.

Proinsulin is a 9390 MW polypeptide of 86 amino acids, that is synthesized in the ß cells of the pancreas and is the precursor molecule for insulin (1, 2, 3). Most proinsulin is converted to insulin and C-Peptide, which are secreted in equimolar amounts into the blood. About 15 % is not converted and is released as proinsulin. The biological activity of proinsulin is only about 10% of Insulin, but the half life of proinsulin is three times as long as insulin.

The level of proinsulin in serum can be a reflection of ß cell status. Both IDDM and NIDDM are characterized by dysfunction of the pancreatic ß cells. Elevated proinsulin levels have been noted at the onset of IDDM and in healthy siblings of IDDM patients. Proinsulin levels may also be increased in patients with established NIDDM.

Increased levels of circulating proinsulin are found in older patients, pregnant or obese diabetics, patients with insulinomas, functional hypoglycemia and hyperinsulinemia, a rare syndrome.

Because the structure of proinsulin is similar to insulin, proinsulin may be detected as immunoreactive insulin in the insulin assay. Immunoreactive insulin levels are generally determined in conventional RIA's, which overestimate the insulin level because the methods use antibodies which crossreact with proinsulin. By calculating the molar ration of proinsulin to true insulin (P/I), a better assessment of ß cell function can be made.

2 PRINCIPLE OF THE TEST

The DRG Proinsulin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Proinsulin molecule. An aliquot of patient sample containing endogenous Proinsulin is incubated in the coated well with enzyme conjugate, which is an anti- proinsulin antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of Proinsulin in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Proinsulin in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. 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.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. 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.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with Anti-Proinsulin antibody (monoclonal).
2. ***Standard (Standard 0-5)***, 6 vials (lyophilized), 1 mL;
   Concentrations: 0 - 2.6 – 6.6 – 16.5 – 33 – 66 pmol/L
   Conversion: 106 pmol/L = 1 ng/mL
   The standards are calibrated against WHO 1st International Standard for Proinsulin IRP (84/611)
   See „Preparation of Reagents“;
   Contain non-mercury preservative.
3. **Control (low and high)**, 2 vials, (lyoph.), 2.0 mL
   see „Preparation of Reagents“
   For control values and ranges please refer to vial label or QC-Datasheet.
   Contain non-mercury preservative.
4. ***Sample Diluent***, 1 vial, 2 mL, ready to use,
   Contains non-mercury preservative.
5. ***Enzyme Conjugate 11X*** **concentrate**, 1 vial, 1.2 mL,
   Anti-Proinsulin antibody conjugated to horseradish Peroxidase;
   see „Preparation of Reagents“.
   Contains non-mercury preservative.
6. ***Conjugate Diluent***, 1 vial, 12 mL, ready to use,
   Contains non-mercury preservative.
7. ***Assay Buffer***, 1 vial, 12 mL, ready to use
   Contains non-mercury preservative.
8. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
9. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
10. ***Wash Solution***, 1 vial, 30 mL (40X concentrated),
    see „Preparation of Reagents“.

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Sample Diluent* for sample dilution is available on request.

4.2 Material required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

4.3 Storage Conditions

When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

4.4 Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Standards
Reconstitute the lyophilized contents of the standard vial with 1.0 mL Aqua dest.
Note: *The reconstituted standards are stable for 3 days at 2 °C - 8 °C. For longer storage freeze at -20 °C.*

Controls
Reconstitute the lyophilized controls with 2.0 mL Aqua dest. each.
Note: *The reconstituted controls are stable for 3 days at 2 °C - 8 °C. For longer storage freeze at -20 °C.*

Wash Solution
Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Enzyme Conjugate
Dilute *Enzyme Conjugate* concentrate 1:11 in *Conjugate Diluent*.
Stability of the prepared Enzyme-Conjugate: 24 h at room temperature.

Example:
If the whole plate is used, dilute 1.2 mL *Enzyme Conjugate* with 12 mL *Conjugate Diluent* to a total volume of 13.2 mL.
If the whole plate is not used at once prepare the required quantity of Enzyme Conjugate by mixing 100 µL of *Enzyme Conjugate 11X* conc. with 1.0 mL of *Conjugate Diluent* per strip (see table below):

No. of strips	*Enzyme Conjugate 11X* conc. (µl)	*Conjugate Diluent* (ml)
1	100	1.0
2	200	2.0
3	300	3.0
4	400	4.0
5	500	5.0
6	600	6.0
7	700	7.0
8	800	8.0
9	900	9.0
10	1000	10.0
11	1100	11.0
12	1200	12.0

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or Heparin-Plasma can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 24 hours at 2 °C - 8 °C prior to assaying.
Specimens held for a longer time (up to two months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µL Serum + 90 µL *Sample Diluent* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **100 µL** of each *Standard*, *Control* and samples with new disposable tips into appropriate wells.
3. Dispense **100 µL** *Assay Buffer* into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Cover the plate with a plate sealer and incubate overnight (16-24 hours) at 4 °C in a humidity chamber.
5. Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with diluted Wash Solution (350 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Dispense **100 µL** diluted *Enzyme Conjugate* into each well.
7. Incubate for **60 minutes** at room temperature (without covering the plate).
8. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted Wash Solution (350 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add **100 µL** of *Substrate Solution* to each well.
10. Incubate for **30 minutes** at room temperature.
11. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
12. Read the OD at **450 ± 10 nm** with a microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 66 pmol/L. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 pmol/L)	0.16
Standard 1 (2.6 pmol/L)	0.25
Standard 2 (6.6 pmol/L)	0.36
Standard 3 (16.5 pmol/L)	0.63
Standard 4 (33 pmol/L)	1.06
Standard 5 (66 pmol/L)	1.82

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

The normal range values observed with DRG Proinsulin ELISA Kit with normal adult males and females are as follows:

	N	Age ± SD	Mean ± SD pmol/L
Post 12-hour Fasting (Plasma)	32	-	4,5 ± 3,8
Post 12-hour Fasting (Serum)	15	32 ± 11	2,5 ± 1,8

Additionally, a glucose tolerance test was performed post 12-hour fasting with 77 healthy children (Age 14 ± 3). Serum was drawn after 12 hours of fasting. Participants were then administered 75 grams of glucose and samples again drawn after 30-120 minutes.

	Mean (± 1SD) pmol/L
Post 12 hour Fasting (Serum)	1,3 (0,5 - 3,5)
30 min. after Glucose administration	6,4 (3,0 - 13,6)
120 min. after Glucose administration	14,8 (6,5 - 33.3)

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.5 pmol/L – 66 pmol/L.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

	Produced Color Intensity Equivalent to Proinsulin
Peptide	**in Serum (pmol/L)**
Proinsulin 32 - 33 split, 500 pmol/L	7.5
Proinsulin 32 - 33 split, 5 pmol/L	0.
Proinsulin Des 31 - 32, 500 pmol/L	4,5
Proinsulin Des 31 - 32, 5 pmol/L	0
Proinsulin 65 - 66 split, 500 pmol/L	275
Proinsulin 65 - 66 split, 5 pmol/L	2,7
Proinsulin Des 64 - 65, 500 pmol/L	266
Proinsulin Des 64 - 65, 5 pmol/L	3,2
Proinsulin 56 - 57 split, 500 pmol/L	375
Proinsulin 56 - 57 split, 5 pmol/L	3,5
Proinsulin Des 57 - 65, 500 pmol/L	271
Proinsulin Des 57 - 65, 5 pmol/L	3,4
Human Insulin, 17000 pmol/L	0
Porcine Proinsulin 2,5 µg/mL	0
Bovine Proinsulin 2,0 µg/mL	0
Rat Proinsulin of Insulin, 160 pmol/L	0
Human C-Peptide, 33000 pmol/L	0
Proinsulin of Somatomedin-C, 10 µg/mL	0
Somatomedin C, 1 µg/mL	0

9.3 Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be < 0.5 pmol/L.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (pmol/L)	CV (%)
1	10	6.97	4.3
2	10	27.2	2.9
3	10	60.3	7.4

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (pmol/L)	CV (%)
1	10	7.32	6.8
2	10	29.6	5.5
3	10	64.7	5.5

9.5 Recovery

Samples have been spiked by adding solutions with known Proinsulin concentrations to patient sera. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added Concentration (pmol/L)	Measured Conc. (pmol/L)	Expected Conc. (pmol/L)	Recovery (%)
1	0	6.8	6.8	
	10	16.9	16.8	101
	30	36.7	36.8	100
	50	52.6	56.8	93
2	0	27.2	27.2	
	10	34.7	37.2	93
	30	55.0	57.2	96
	50	73.1	77.2	95

9.6 Linearity

Patient sera were serially diluted with sample diluent. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Dilution	Measured Conc. (pmol/L)	Expected Conc. (pmol/L)	Recovery (%)
6	None	72.65	72.65	
	1:2	36.88	36.33	101.5
	1:4	18.72	18.16	103.0
	1:8	9.33	9.08	102.7
	1:16	4.63	4.54	101.9
7	None	61.86	61.86	
	1:2	31.01	30.93	100.3
	1:4	16.02	15.47	103.6
	1:8	8.06	7.73	104.1
	1:16	4.12	3.87	106.5

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

High concentrations of Haemoglobin, Bilirubin and Triglyceride may influence the assay results.
Sodium azide interferes with the assay result.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Proinsulin in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 6000 pmol/L.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES / LITERATURE

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