TECOmedical Group

A EUROBIO SCIENTIFIC COMPANY

TECO®

Human Intact Proinsulin ELISA

Human Intact Proinsulin ELISA

Instructions for Use
English

Cat. No. TE1012

UDI-DI 7640146270016

CE

TE1012_AA-E_01/2023

always your partner

Symbol Description

REF
TE1012

LOT
Lot number

UDI-DI
Unique Device Identifier

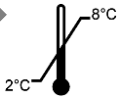
IVD
In Vitro Diagnostics

Manufacturer

Read electronic kit instructions


96
96 tests


Expiry date



Storage temperature


Consult Instructions for Use

CE

Professional Use only

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CONT Reagents and Materials Supplied:

SYMBOL	DESCRIPTION	FORMAT
1	Intact Proinsulin Antibody Coated Microtiter Plate 12 strips of 8 wells (96 breakable wells in total), in a frame, Ready to use	1 plate
2	Blocking Buffer Ready to use	1 x 1,5 ml
3	Antibody-HRP Conjugate Ready to use 	1 x 11 ml
4	TMB Substrate Ready to use	1 x 25 ml
5	Wash Solution 10 times concentrated 	1 x 40 ml
6	Stop Solution – 0.5 M H_2SO_4 0.5 M sulfuric acid, ready to use 	1 x 15 ml
A	Standard A 0 pmol/L, lyophilised	2 x 3,0 ml
B	Standard B lyophilised, Concentration see data sheet	1 x 1,0 ml
C	Standard C lyophilised, Concentration see data sheet	1 x 1,0 ml
D	Standard D lyophilised, Concentration see data sheet	1 x 1,0 ml
E	Standard E lyophilised, Concentration see data sheet	1 x 1,0 ml
F	Standard F lyophilised, Concentration see data sheet	1 x 1,0 ml
L	Control 1 lyophilised, Range see data sheet	1 x 1,0 ml
H	Control 2 lyophilised, Range see data sheet	1 x 1,0 ml
	**eIFU indicator** Login address for electronic kit instructions	

Storage
Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

Instructions for Use
The TECO® Human Intact Proinsulin ELISA is a sensitive ”two-site” sandwich enzyme-linked immunosorbent assay for the quantitative determination of intact Proinsulin in human plasma and serum.

Clinical Use
Proinsulin is produced in the pancreatic β-cells and is normally further processed to insulin and C-peptide. It is only seen in low concentrations in the plasma of healthy subjects. Insulin resistance (IR) or hyperglycemia causes increased insulin secretion and finally a secretion disorder. Intact proinsulin levels in plasma then increase while insulin levels decrease. Elevated fasting intact proinsulin is a specific biomarker for β-cell dysfunction and IR and independent risk factor for cardiovascular disease.

In clinical practice, fasting morning intact proinsulin can be used as highly specific indicator of clinically relevant insulin resistance and β-cell dysfunction, the underlying cause of type 2 diabetes. Levels can be used to serve as the basis for the selection of an insulin resistance therapy, and to monitor the therapeutic effect on ß-cell dysfunction. Patients with elevated fasting intact proinsulin levels should be regarded and treated as insulin resistant and possibly prediabetic, in order to reduce the risk for further cardiovascular damage.

When used during the oral glucose tolerance test, the 2-hour intact proinsulin levels are highly predictive of future development of type 2 diabetes, even before glucose, HbA1c and insulin changes are detectable. In fact, 2-hour intact proinsulin test predicts type 2 diabetes up to 4 years before clinical manifestation.

Elevated fasting intact proinsulin levels may also be seen in patients with insulinoma, or prior to manifestation of type 1 diabetes.

References

1 Vangipurapu J, Stančáková A, Kuulasmaa T, Kuusisto J, Laakso M. Both fasting and glucose-stimulated proinsulin levels predict hyperglycemia and incident type 2 diabetes: a population-based study of 9,396 finnish men. PLoS One 2015; 10:e0124028

2 Pfützner A, Hermanns I, Ramljak FS, Demircik F, Pfützner AH, Kann PH, Weber MM. Elevated Intact Proinsulin Levels During an Oral Glucose Challenge Indicate Progressive ß-Cell Dysfunction and may be Predictive for Development of Type 2 Diabetes. J Diabetes Sci Technol J Diabetes Sci Technol. 2015; 9:1307-12.

3 Pfützner A, Forst T. Elevated intact proinsulin levels are indicative of Beta-cell dysfunction, insulin resistance, and cardiovascular risk: impact of the antidiabetic agent pioglitazone. J Diabetes Sci Technol. 2011 May 1;5(3):784-93. Review.

4 Pfützner A, Sachsenheimer D, Lier A. Erhöhtes intaktes Proinsulin als früher Hinweis auf einen zukünftigen Typ 2 Diabetes. Increased Intact Proinsulin as an early indication of a future type 2 diabetes. Diabetes Stoffw Herz 2018; 27:69-73.

Assay Principle

The TECO® human Proinsulin ELISA is a sensitive two-site sandwich enzyme-linked immunosorbent assay. The microtiter plates are coated with a monoclonal antibody (S2) specific for an epitope at the C-peptide/insulin A chain junction. The antibody is able to bind intact proinsulin, des (31,32)-proinsulin and split (32,33)- proinsulin but not insulin, C-peptide and the other “des” and “split” forms.

First, a blocking buffer is added to the allocated wells. An aliquot of patient sample is then added to the wells. After incubation, the wells are washed to remove unbound antibody and other serum compounds. In a second incubation time, an enzyme labelled monoclonal proinsulin antibody is added. This antibody is specific for the epitopes at insulin β chain/C-peptide junction. S53 is able to bind to intact proinsulin, des (64,65)- proinsulin and split (65,66)- proinsulin but not insulin, C-peptide and other “des” and “split” forms. The combination of these two monoclonal antibodies has the ability to detect only the intact human proinsulin.

After washing, the remaining bound enzyme activity is measured by adding a chromogenic substrate. The intensity of color development is proportional to the concentration of proinsulin in the patient sample.

Materials Required and not Supplied

- Pipettes capable of dispensing 50 µl, 100 µl, 150 µl and 300 µl
- Graduated cylinders for reconstituting or diluting reagents
- Manual Aspiration System and multi-channel pipette or automatic washer
- Aqua dest
- Vortex mixer
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 and 405 nm and with 590-650 for reference.
- ELISA plate shaker (400 rpm) (orbital shaker)
- Software package for data reduction and analysis

Warnings and Precautions

This kit is intended for in vitro use by professional persons only.

Follow the instructions carefully.

Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to “Materials Safety Data Sheet” for more detailed safety information.

Material of human origin used in the preparation of this kit has been tested and found nonreactive for HIV-1 and HIV-2 as well as for HCV antibodies and HbsAg but should, nonetheless, be handled as potentially infectious.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

1 For in vitro diagnostic use.
2 Treat all specimen samples as potentially biohazardous material. Follow General Precautions when handling contents of this kit and any patient samples.
3 Disposal of containers and unused contents should be done in accordance with federal and local requirements.
4 Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5 Store assay reagents as indicated.
6 Do not use coated strips if pouch is punctured.
7 Test each sample in duplicate.

8 Use of multi-channel pipettes or repeat pipettors is recommended to ensure the timely delivery of liquids

9 0,5 M sulfuric acid is caustic and can cause severe burns.

10 Handle TMB, Wash buffer Buffer with care.
Do not ingest. Avoid contact with skin, eyes, or clothing. Should there be any contact, wash with water. If ingested, call a physician.

Preparation of Reagents

1 Microtiter plate coated with a proinsulin specific Antibody
12 strips of 8 wells (96 breakable wells in total) in a frame and sealed in a foil bag. Fit strip wells firmly into the frame. After opening, immediately return any unused wells to the original foil package and seal.
Store at 2–8 °C until expiration date.

A Proinsulin 0 Standard
2 vials of 0 Standard, lyophilized. Reconstitute each vial with 3 ml Aqua dest. Blue coded. After reconstitution, keep the standard at -20 °C (freeze/thaw: max 2 times). Stable for 2 months.
Store lyophilized at 2–8 °C until expiration date.

B-F Standards
5 vials of lyophilized Standard. Reconstitute each vial with 1 ml of distilled water. Blue coded. After reconstitution, keep the standard at -20 °C (freeze/thaw: maximum 2 times). Stable for 2 months. For the exact value, refer to the data sheet included. The Standards are standardized against the WHO International Reference reagent 09/296.
Store lyophilized at 2–8 °C until expiration date.

L Control 1
1 vial of lyophilized control. Reconstitute with 1 ml of distilled water. Blue coded. After reconstitution, keep the control serum at -20 °C (freeze/thaw: maximum 2 times). Stable for 2 months. For the exact value, refer to the data sheet included.
Store lyophilized at 2–8 °C until expiration date.

H Control 2
1 vial of lyophilized control. Reconstitute with 1 ml of distilled water. Blue coded. After reconstitution, keep the control serum at -20 °C (freeze/thaw: maximum 2 times). Stable for 2 months. For the exact value, refer to the data sheet included.
Store lyophilized at 2–8 °C until expiration date.

2 Blocking Buffer
1 vial of 1.5 ml of murine IgG in phosphate buffer. Ready to use. Store at 2–8 °C until expiration date.
Blocking Buffer Working solution
Either prepare the necessary volume to use immediately, or the total volume and store at -20 °C.
1 part Blocking Buffer + 4 parts 0 Standard (e.g. mix 1.2 ml Blocking Buffer **2** + 4.8 ml Proinsulin 0 Standard **A**). Stable for 2 months if stored at -20 °C. (Maximum 2 freeze/thaw cycles).

3 Antibody-HRP Conjugate
1 vial of 11 ml of anti-human proinsulin conjugated to horseradish peroxidase (HRP). Ready to use.
Store at 2–8 °C until expiration date.

4 TMB Substrate
1 vial of 25 ml of Tetramethylbenzidine in citrate-phosphate buffer and DMSO. Ready for use.
Store at 2–8 °C until expiration date.

5 Wash Solution
1 vial of 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.
Store undiluted at 2–8 °C until expiration date.

6 Stop Solution – 0.5 M H_2SO_4
1 vial of 15 ml of 0.5M H_2SO_4 Ready to use.
Store at 2–8 °C until expiration date.

Preparation and Stability of Serum Samples

Caution
In order to use the assay's results for conclusions about the function of the ß-cells, it is recommended to test fasting morning samples.

Sample Type
Fasting blood samples. Human serum or plasma. Due to higher stability, EDTA or heparin plasma samples are preferred to serum samples.

Plasma
The sample collection can take place in HbA1C–tubes. These samples are stable at room temperature and should be centrifuged within 48 hours. Plasma should be used in the assay or can be stored in aliquots, stable > 2 years at -20 °C. Avoid repeated freeze/thaw cycles.

Serum
Centrifuge whole blood within 4 hours. Proteases degrade intact proinsulin in serum, do not store longer than 1 day at 2–8 °C. Serum should be used in the assay or can be stored in aliquots at -20 °C. For further information about sample stability see: Pfützner et al. Clinical and Laboratory Evaluation of a New Specific ELISA for Intact Proinsulin. Clin Lab 51:,243-249, 2005.

Assay Procedure

NOTE
In order to obtain an optimal differentiation in the cut-off range (7 pmol/l) it is recommended to use Standards A till E (0~60 pmol/l) and to measure the absorption at 450 nm with a reference filter of 590–650 nm.
A second measurement of Standards A till F (0~140 pmol/l) can be done at 405 nm with a reference filter of 590–650 nm.

Allow all reagents to stand at room temperature (20–25 °C) for at least 30 minutes.

1 Prepare the frame and the required number of coated strips 1. Allocate the wells of the Microtiter plate for Standards, Controls and samples.

2 Pipette 50 µl of Blocking Buffer Working solution 2 directly into the bottom of the wells.

3 Pipette 50 µl of each Standards A till F, Controls 1 and 2 (L and H) and samples into the corresponding wells.

4 Cover the strips and incubate for 60 minutes at room temperature (20–25 °C) on an orbital shaker (400 rpm).

5 After incubation, aspirate the wells by using a plate washer or manually decant by inverting the plate. Wash the wells 3 x with 300 µl diluted washing buffer. After the last wash cycle tap the inverted wells gently on a dry absorbent surface to remove excess wash solution.

6 Add 100 µl of HRP Conjugate 3 into the wells.

7 Cover the strips and incubate for 60 minutes at room temperature (20–25 °C) on an orbital shaker (400 rpm).

8 Repeat wash step 5.

9 Pipette 150 µl of TMB Substrate 4 into the wells and incubate for 15–25 minutes at room temperature on an orbital shaker (400 rpm).

10 Add 100 µl of Stop Solution 6 into the wells, shake for 5 seconds on a plate shaker and read the absorbance within 15 minutes.

11 Read the absorbance of the wells (450, 405 nm). Reference filter at 590–650 nm.

12 If dilution of samples is required, dilution should be done with zero standard (recommended dilution 1:4).

Protocols for the different automatic ELISA systems are available.

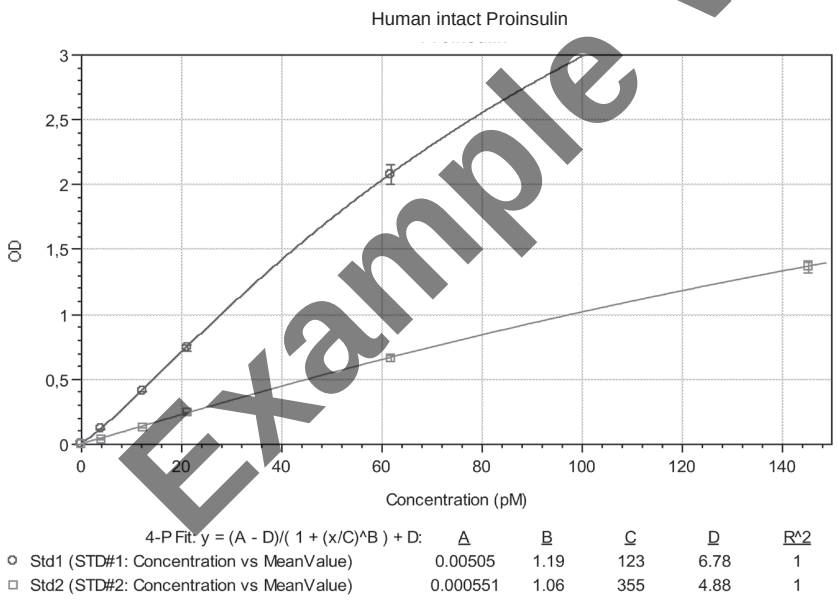
Result Analysis

A standard curve can be established by plotting standard concentration on the x-axis (linear scale) against the absorbance of the standards on the y-axis (linear scale). The intact proinsulin concentrations in patient sera can then be read off the standard curve. A 4-parameter curve fit should be used for automatic data reduction.

TYPICAL RESULTS 450 AND 405 nm

(Example only, not for use in calculation of actual results)

STANDARD	pmol/l	Extinction at 450 nm	Extinction at 405 nm
A	0	0.003	0.002
B	3.9	0.118	0.04
C	12.3	0.409	0.133
D	21.1	0.746	0.238
E	61.8	2.077	0.663
F	145.3	-	1.367
L-control 1	15.2 (9.8 - 20.5)	0.522	0.168
H-control 2	39.2 (29.4 - 48.9)	1.432	0.458



Reference Values*

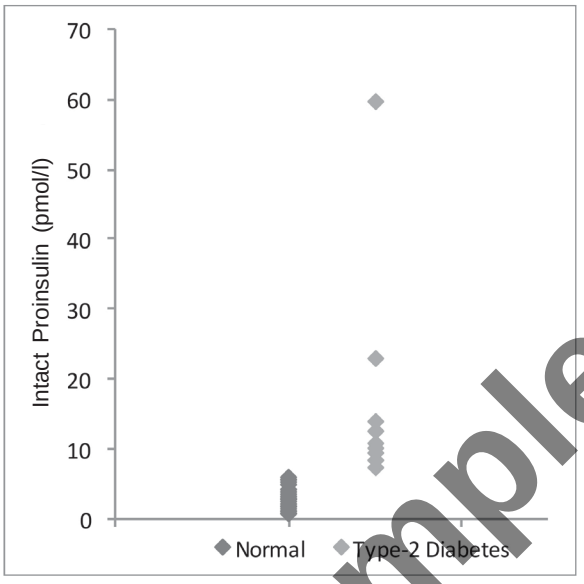
Normal values (N=32) for intact proinsulin in EDTA plasma were obtained with the Human Intact Proinsulin ELISA kit from a group of healthy men and women.

- Mean value: 2.67 ± 1.54 SD pmol/l

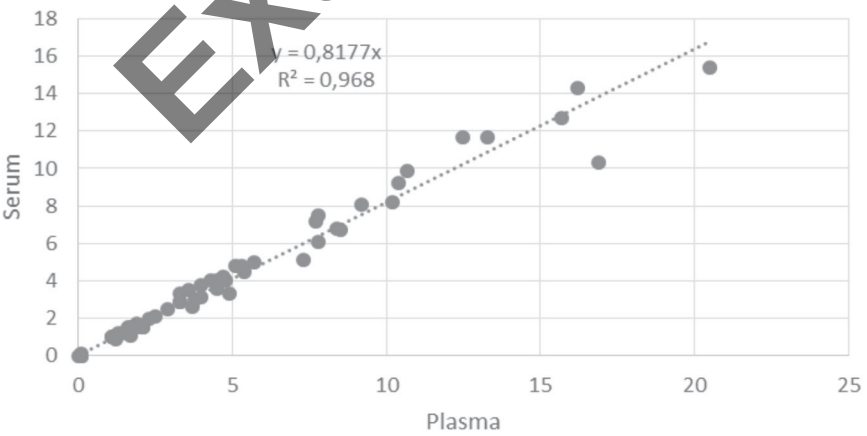
HEALTHY SUBJECTS (N=32) VERSUS TYP2 DIABETES PATIENTS – HOMA SCORE > 2 (N=11)
Fasting values:

- values ≤ 7 pmol/L (WHO 9/296) are considered normal.
- values > 7 pmol/L (WHO 9/296) suggest progressive β-cell dysfunction, insulin resistance and possibly type 2 (pre)diabetes. It is also a high-risk indicator for cardiovascular disease.

Healthy persons



Intact Proinsulin Correlation Serum - EDTA Plasma
Serum values are 15-20% lower in comparison to EDTA Plasma (N=58)



* Cut-off value 11pmol/l in literature references - previous Assay version with WHO 84/611calibration.

Intact Proinsulin – Glucose tolerance testing

Type 2 diabetes mellitus is a complex disease, which usually presents with a genetically driven β-cell dysfunction, visceral obesity and a metabolic insulin resistance. Intact proinsulin is an indicator of severe β-cell dysfunction and has been demonstrated to be an indirect biomarker for insulin resistance when elevated in the fasting morning state [1-3]. In the majority of the cases, severe β-cell dysfunction with increased intact proinsulin secretion is preceding the final clinical onset of type 2 diabetes. Up to 30 % of insulin resistant and (pre)diabetic patients remain undiagnosed due to normal blood glucose and HbA1c levels. Early detection of insulin resistance is very important, as many type 2 diabetes patients already show irreversible cardiovascular damages during the first clinical diagnosis of their disease with high risk of cardiovascular events. Still, 75 % of T2D patients die of cardiovascular events, whereas this is only true for 35 % of patients with type 1 diabetes.

CASE STUDY

This situation becomes most apparent during an oral glucose tolerance test (OGTT). Twenty normal individuals (10 male, 10 female, age 29-83) were subjected to OGTT [4]. Glucose, HbA1c, insulin and intact proinsulin levels were measured at time points 0, 1 hour and 2 hours after oral administration of 75 grams of glucose. Intact proinsulin was measured using TECOmedical Intact Proinsulin ELISA.

Four patients showed remarkable results, their glucose, insulin and HbA1c levels were normal at time point 0 and 2 hours, however intact proinsulin was also normal at time point 0 but significantly increased (> 7 pmol/L)at time point 2 hours. These patient cases are described in further detail below. All 4 patients developed clinically manifest type 2 diabetes 3-4 years after the initial OGTT. All others (16) showed normal values for all markers and did not develop disease.

CASE 1

Male, 83 years old, (BMI: 29.5 kg/m2) with controlled hypertension and a family history of T2D (mother). During OGTT in 2011, HbA1c was normal (5.7 %). OGTT results were as follows:

	GLUCOSE	INTACT PROINSULIN
Normal value	80-120 mg/dl	< 7 pmol/l
0 h	104	1.56
2 h	67	11.94

End 2014, T2D was clinically confirmed in this patient, in 2015 is was under control with Metformin medication.

CASE 2

Female, 83 years old, (BMI: 28.5 kg/m2) with controlled dyslipidemia and hypertension, no family history of T2D. Both parents had died 25 years ago from myocardial infarction. During OGTT in 2011, HbA1c was normal (5.5 %). OGTT results were as follows:

	GLUCOSE	INTACT PROINSULIN
Normal value	80-120 mg/dl	< 7 pmol/l
0 h	86	2.36
2 h	126	10.21

Patient was subsequently followed with intervals of 6 months. Early 2015 fasting Intact Proinsulin suddenly increased to 12,3 pmol/L. T2D manifested during another OGTT (glucose values 0: 123 mg/dL and 2 H: 196 mg/dL). T2D was successfully treated with medication.

CASE 3
Female, 46 years old, (BMI: 34.2 kg/m2) with manifest obesity. Family history of dyslipidemia and hyper-uricemia and T2D in both living parents. During OGTT in 2011, HbA1c was normal (5.6 %). OGTT results were as follows:

	GLUCOSE	INTACT PROINSULIN
Normal value	80-120 mg/dl	< 7 pmol/l
0 h	94	1.88
2 h	72	12.45

Patient was subsequently followed regularly. Although patient was able to reduce weight by 15 kg, clinically manifest T2D was diagnosed end of 2013 during another OGTT (glucose values 0: 105 mg/dL and 2 H: 211 mg/dL).

CASE 4
Male, 29 years old, (BMI: 38.3 kg/m2) with morbid obesity. Father had died at age 48 from myocardial infarction, no record of disease with the mother. During OGTT in 2011, HbA1c was normal (5.6 %). OGTT results were as follows:

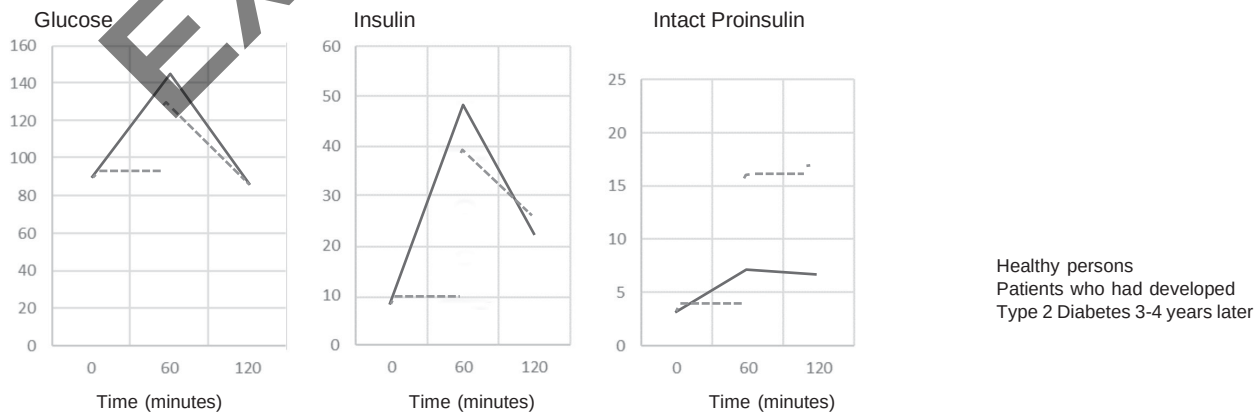
	GLUCOSE	INTACT PROINSULIN
Normal value	80-120 mg/dl	< 7 pmol/l
0 h	86	3.55
2 h	92	11.94

Patient was subsequently followed regularly. Attempts to reduce weight were unsuccessful. Clinically manifest T2D was diagnosed end of 2014 during another OGTT (glucose values 0: 117 mg/dL and 2 H: 243 mg/dL). After consultation, patient agreed to bariatric surgery and a gastric band. Weight loss was over 30 kg in 6 months (BMI: 26.2 kg/m^2).

Conclusions

In all 4 cases, later development of type 2 diabetes was already predicted 3-4 years earlier with increased Intact Proinsulin 2-hour levels during OGTT. All other 16 patients were also closely followed over 6 more years, no T2D was diagnosed. The figure below shows the OGTT results for both patient groups at initial OGTT.

Intact Proinsulin indicates type 2 pre-diabetes before glucose changes are detectable. It can predict type 2 diabetes development up to 4 years before clinical diagnosis. Glucose, insulin and HbA1c cannot detect prediabetes and predict later T2D development.



Glucose, insulin and intact proinsulin profiles in OGTT for patients who developed T2D within 4 years from this test. Results are compared to healthy persons who did not develop T2D.

> Only intact proinsulin predicts later T2D development.
> (glucose in mg/dL; insulin in µU/mL); intact proinsulin in pmol/L).

CLINICAL INTERPRETATION OF INTACT PROINSULIN LEVELS DURING oGTT:

- values ≤ 7 pmol/L (WHO 09/296) are considered normal and suggest normal β-cell function and no risk for type 2 diabetes and cardiovascular disease.
- values > 7 pmol/L (WHO 09/296) indicate progressive β-cell dysfunction and insulin resistance and are highly predictive of development of type 2 diabetes within 4 years.

Test Performance

STANDARD

This test is standardized against the International Standard for Intact Proinsulin (WHO 09/296), National Institute for Biological Standards and Control, Hertfordshire, England

PRECISION (INTRA ASSAY)

N = 6	MEAN VALUE pmol/l	%CV
Sample 1	5.38	2,2
Sample 2	9.31	1,8

PRECISION (INTER ASSAY)

N = 5	MEAN VALUE pmol/l	%CV
Sample 1	5.27	4.0
Sample 2	9.06	1.8
Sample 3	16.68	3.1
Sample 4	32.46	1.7

DETECTION LIMIT

The kit zero standard was assayed 10 times and the mean and standard deviation were calculated. The lower detection limit at +2 standard deviations is 0.15 pmol/L.

- LLOQ = 0.49 pmol/L
- ULOQ = highest standard 450 or 405 nm

RECOVERY TEST

SERUM SAMPLE	PROINSULIN ADDED pmol/l	EXPECTED pmol/l	OBSERVED pmol/l	RECOVERY(%)
Serum 1	0 10	3.98 13.98	3.98 14.80	100.00 109.40
Serum 2	0 10	13.34 23.34	13.34 23.32	100.00 106.20
Serum 3	0 10	3.68 13.68	3.68 14.19	100.00 107.00
Serum 4	0 10	4.62 14.62	4.62 12.53	100.00 88.80
Serum 5	0 10	5.01 15.01	5.01 15.37	100.00 106.30

SERUM SAMPLE	PROINSULIN ADDED pmol/l	EXPECTED pmol/l	OBSERVED pmol/l	RECOVERY(%)
Serum 1	0 10	51.10 61.10	51.10 62.05	100.00 110.90
Serum 2	0 10	54.13 64.13	54.13 63.24	100.00 107.80
Serum 3	0 10	47.15 57.15	47.15 56.14	100.00 107.20
Serum 4	0 10	36.85 46.85	36.85 46.66	100.00 108.20
Serum 5	0 10	38.38 48.38	38.38 47.74	100.00 107.30

DILUTION TEST

SERUM SAMPLE	DILUTION FACTOR	EXPECTED pmol/l	OBSERVED pmol/l	RECOVERY(%)
Serum 1	1	3.98	3.98	100.00
	2	1.99	1.93	97.00
	4	1.00	0.99	99.50
Serum 2	1	13.34	13.34	100.00
	2	6.67	6.97	104.50
	4	3.34	3.90	116.90
Serum 3	1	3.68	3.68	100.00
	2	1.84	1.84	100.00
	4	0.92	0.93	101.10
Serum 4	1	4.62	4.62	100.00
	2	2.31	2.58	111.70
	4	1.16	1.31	113.40
Serum 5	1	5.01	5.01	100.00
	2	2.51	2.52	100.60
	4	1.25	1.45	115.80

SERUM SAMPLE	DILUTION FACTOR	EXPECTED pmol/l	OBSERVED pmol/l	RECOVERY(%)
Plasma 1	1	51.50	51.10	100.00
	2	25.55	29.44	115.20
	4	12.78	15.78	123.50
Plasma 2	1	54.13	54.13	100.00
	2	27.07	29.87	110.40
	4	13.53	15.97	118.00
Plasma 3	1	47.15	47.15	100.00
	2	23.58	26.63	113.00
	4	11.79	15.01	127.30
Plasma 4	1	36.85	36.85	100.00
	2	18.43	19.64	106.60
	4	9.21	11.37	123.40
Plasma 5	1	38.38	38.38	100.00
	2	19.19	21.30	111.00
	4	9.60	11.31	117.90

INTERFERENCE

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 a HAMA 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. Samples from LADA patients can contain high HAMA concentrations and other non-specific antibodies.

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	5000 pmol/L
Des (64,65)-Proinsulin*	200 pmol/L
Split (65,66)-Proinsulin	1000 pmol/L

* not present in Serum and Plasma samples

REMARK

The data quoted in this instruction should be used for guidance only. It is recommended that each laboratory includes its own panel of control samples in the assay. In order to follow GLP guidelines, each laboratory should establish its own normal and pathological ranges for Intact Proinsulin levels.

TECO® human Intact Proinsulin

ASSAY PROCEDURE – QUICK GUIDE

· Bring samples and reagents to room temperature.
· Reconstitute the 2 vials Proinsulin 0 Standard A with 3 ml Aqua Dest each.
· Prepare Blocking Buffer Working Solution: 1 part Blocking Buffer 2 plus 4 parts 0 Standard A (e.g. mix 1.2 ml Blocking Buffer + 4.8 ml Proinsulin 0 Standard. Store at -20 °C.
· Prepare Washing Buffer: Take 1 vial (40 ml) of concentrated Wash Buffer 5 and complete until 400 ml with Aqua dest.
· Reconstitute lyophilised Standards B till F and controls L and H with 1 ml Aqua dest each.

Prepare the required number of Assay Strips 1

Pipette **50 µl** Blocking Buffer Working Solution into each well

Pipette **50 µl** Standards A till F , Controls L and H and Samples

Incubate **60 min** at 20–25 °C on a Rotator at 400 rpm

Aspirate and wash **3 x** with **300 µl** Wash Buffer, aspirate and tap the inverted wells gently on a clean dry absorbent surface

Pipette **100 µl** HRP Conjugate 3 into each well

Incubate **60 min** at 20–25 °C on a Rotator at 400 rpm

Aspirate and wash **3 x** with **300 µl** Wash Buffer, aspirate and tap the inverted wells gently on a clean dry absorbent surface

Add **150 µl** TMB Substrate 4 into each well

Incubate **15-25 min** at 20–25 °C on a Rotator at 400 rpm

Add **100 µl Stop Solution** 6 into each well and shake for 5 seconds

Measure the absorbance at 450 nm, Standard A till E
Measure the absorbance at 405 nm, Standard A till F
(Quantification software, 4-parameter fit:
y = (A-D)/(1+(x/C)^B)+D) Reference measurement should be performed at 590-650 nm

Please read Kit Instruction before using the Quick Guide.