

TECO® *Proinsulin*

Human Intact Proinsulin ELISA

Instructions for Use
English

Catalogue No. TE1011
For Research Use Only.

always your partner

Symbol Description



Kit Instructions



Lot Number



Expiry Date



Storage Temperature



Manufacturer



Caution: read instructions



Caution: irritant



Tests

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- Certified Management System
- EN ISO 9001
- EN ISO 13485

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TECO® human Intact Proinsulin ELISA-Kit

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	2 x 15 ml
5	Wash Solution 10 times concentrated	1 x 40 ml
6	Stop Solution – 0.5 M H₂SO₄ 0.5 M sulfuric acid, ready to use	 1 x 15 ml
A	Standard A 0 pmol/L, lyophilized	2 x 3.0 ml
B	Standard B lyophilized, Concentration see data sheet	1 x 1.0 ml
C	Standard C lyophilized, Concentration see data sheet	1 x 1.0 ml
D	Standard D lyophilized, Concentration see data sheet	1 x 1.0 ml
E	Standard E lyophilized, Concentration see data sheet	1 x 1.0 ml
F	Standard F lyophilized, Concentration see data sheet	1 x 1.0 ml
L	Control 1 lyophilized, Range see data sheet	1 x 1.0 ml
H	Control 2 lyophilized, Range see data sheet	1 x 1.0 ml
	Kit Instruction	1 x

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 kit is a sensitive "two-site" sandwich enzyme-linked immunosorbent assay for the quantitative determination of intact Proinsulin in human plasma and serum.

Background

Proinsulin is produced in the pancreatic β -cells and is normally further processed to insulin and C-peptide. An increase in the insulin demand, as provided by insulin resistance in later stages of type 2 diabetes mellitus, can result in increased expression of proinsulin into the blood. Intact proinsulin is rapidly degraded, but is considered to be an independent cardiovascular risk factor. The intact molecule and its degradation products are known to block fibrinolysis because of plasminogen-activator inhibitor (PAI-1) stimulation.

References

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Role of intact proinsulin in diagnosis and treatment of type 2 diabetes mellitus.
Diab Technol Ther 6: 405-412, 2004.
- 4 Pfützner A, Kunt T, Hohberg C, Mondok A, Pahler S, Konrad T, Lübben G, Forst T.
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- 5 Piovesan A, Pia A, Visconti G, Terzolo M, Leone A, Magro G, Cesario F, Borretta G.
Proinsulin-secreting neuroendocrine tumor of the pancreas.
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- 6 Paule Housa et al.
First direct assay for intact human proinsulin.
Clinical Chemistry 44: 7, 1514-1519, 1998.
- 7 Hanley AJ et al.
Increased proinsulin levels and decreased acute insulin response independently predict the incidence of type 2 diabetes in the insulin resistance atherosclerosis study.
Diabetes 51: 1263-1270, 2002.
- 8 Kricka L.
Human anti-animal antibody interferences in immunological assays.
Clinical Chemistry 45: 942-956, 1999.
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Intact and total proinsulin: new aspects for diagnosis and treatment of type-2-diabetes.
Clinical Lab. 50 (2004) 567-573
- 10 Langenfeld M, Forst T, Standl E, Strotmann HJ, Luebben G, Pahler S, Kann P, Pfuetzner A.
IRIS II Study: Sensitivity and Specificity of Intact Proinsulin, Adiponectin and the Proinsulin/Adiponectin Ratio as Markers for Insulin Resistance.
Diab. Technol. Ther. 6 (2004) 836-843

Assay Principle

The TECO® human Proinsulin ELISA Kit 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 or bound enzyme activity is measured by adding a chromogenic substrate. The intensity of colour development is proportional to the concentration of proinsulin in the sample.

Materials Required and not Supplied

- Pipettes capable of dispensing 50 μ l, 100 μ l, 150 μ l and 300 μ l
- Graduated cylinders for reconstituting or diluting reagents
- Manual Aspiration System and multi-channel pipette or automatic washer
- Distilled water
- 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 research 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 non-reactive 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 Research Use Only. Not for use in diagnostic procedures.
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. a) 0.5 M sulfuric acid is caustic and can cause severe burns.
b) handle TMB and Blocking 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.
10. Sodium azide is used as a preservative. Incidental contact or ingestion of buffer solutions containing sodium azide can cause irritation of skin, eyes or mouth. Sodium azide may react with lead, copper or brass plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azides build-up.

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 distilled water. Blue coded. After reconstitution, keep the standard at -20 °C (freeze/thaw: max 2 times). Stable for 2 months. Preservative: Merthiolate. Store lyophilized at 2–8 °C until expiration date.

B

Standards

thru

F

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 1st International Reference reagent IRR 84/611. Preservative: Merthiolate. 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. Preservative: Merthiolate. 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. Preservative: Merthiolate. 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 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). Preservative: Merthiolate.

3 Antibody-HRP Conjugate

1 vial of 11 ml of anti-human proinsulin conjugated to horseradish peroxidase (HRP). Ready to use. Preservatives: Neomycin and Merthiolate. Store at 2–8 °C until expiration date.

4 TMB Substrate

2 vials of 15 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. Preservatives: Streptomycin sulfate and Amphotericin. 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 H₂SO₄

1 vial of 15 ml of 0.5M H₂SO₄. 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 β-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 lower range (11 pmol/l) it is recommended to use Standards **A** thru **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** thru **F** (0~100 pmol/l) can be done at 405 nm with a reference filter of 590–650 nm to evaluate the entire range of the assay.

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** thru **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 ml 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.

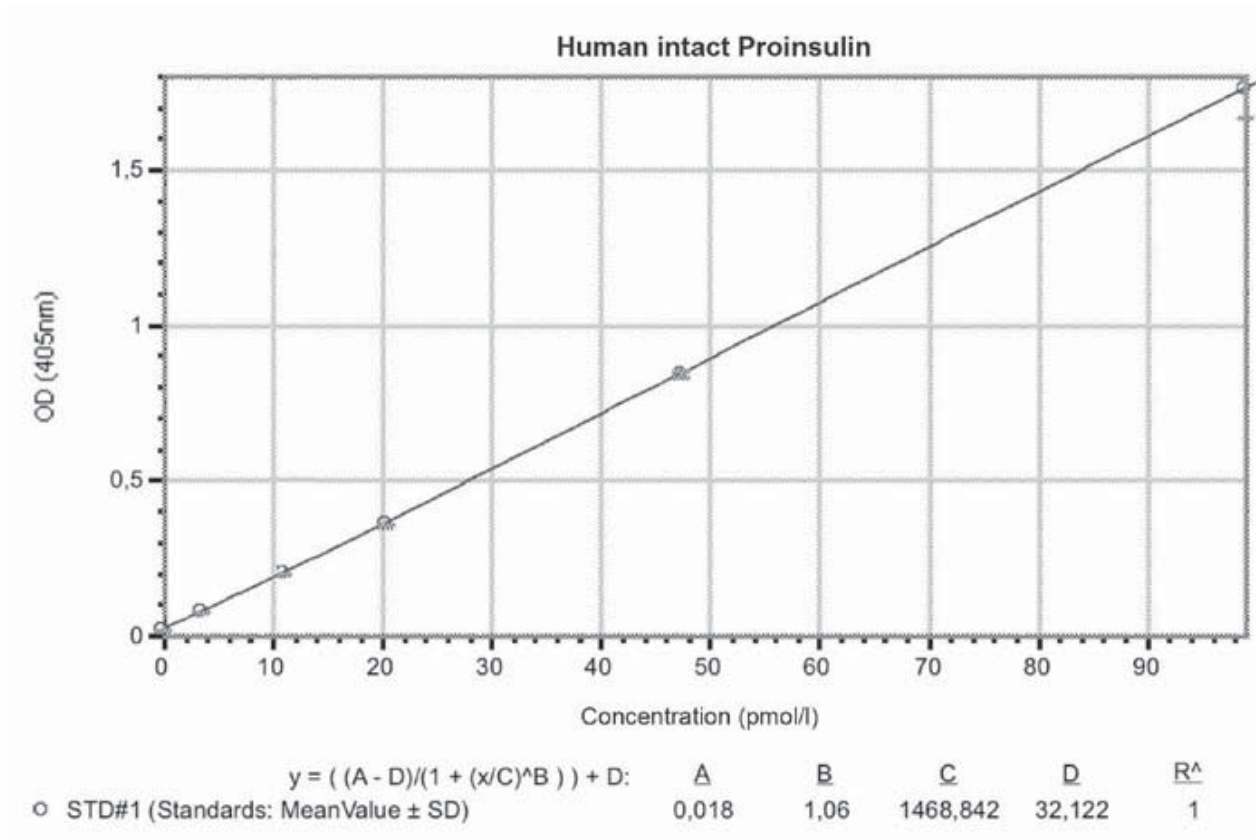
Result Analysis

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 nm

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

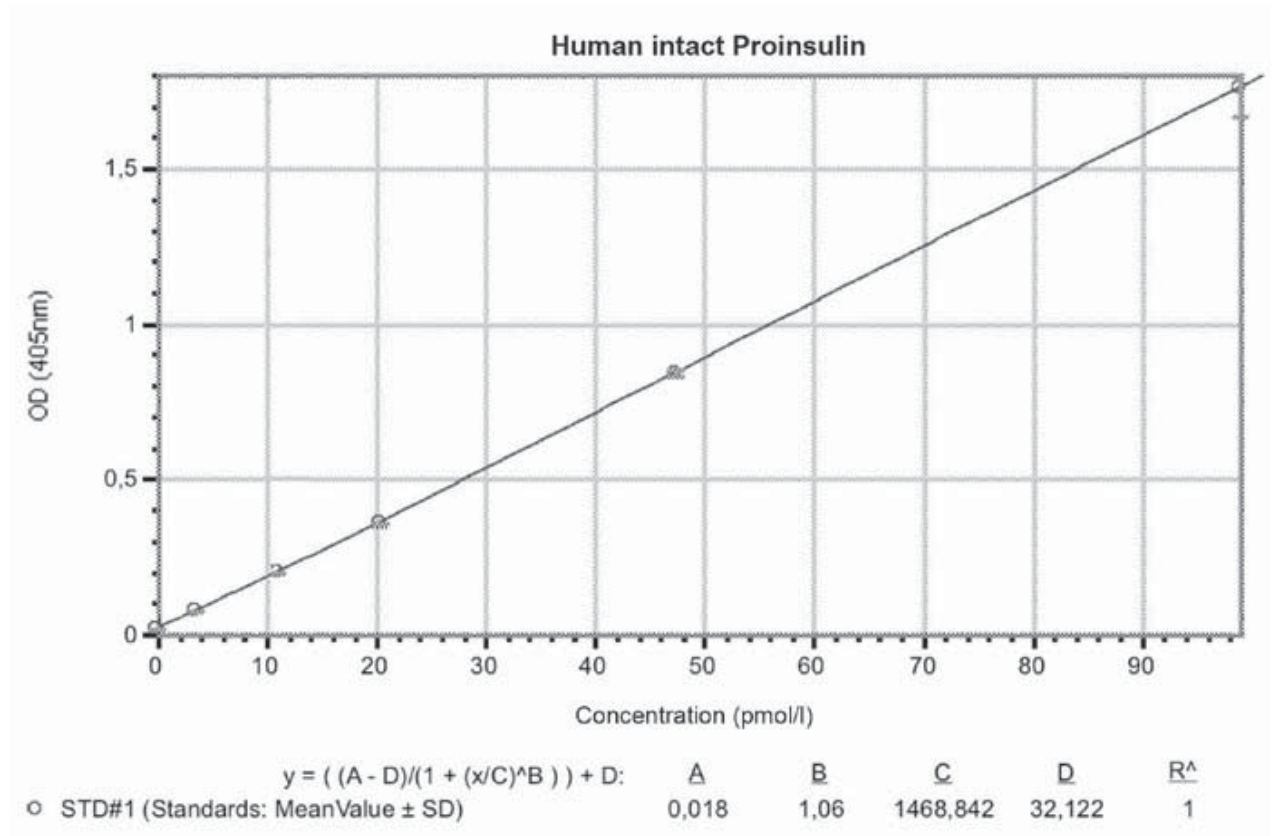
Standard	Absorbance at 450 nm	pmol/l
A	0.017	0.0
B	0.232	4.5
C	0.709	12.9
D	1.296	25
E	2.776	60
F	–	117
L-Control 1	0.896	16.8(13.4–20.2)
H-Control 2	1.650	32.4(25.9–38.8)



Typical Results 405 nm

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

Standard	Absorbance at 405 nm	pmol/l
A	0.017	0.0
B	0.085	4.5
C	0.233	12.9
D	0.416	25
E	0.997	60
F	1.985	117
L-Control 1	0.290	16.8(13.4–20.2)
H-Control 2	0.540	32.4(25.9–38.8)



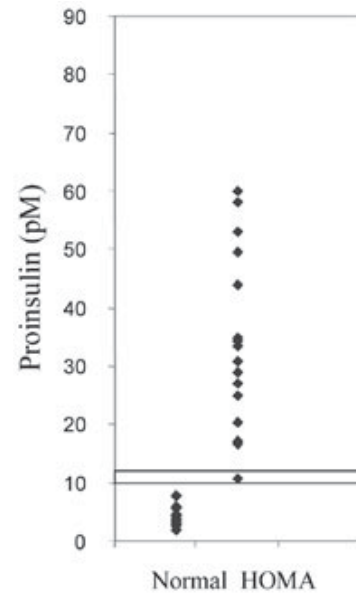
For each assay, the results of the controls must be within the target range indicated for every lot. The QC protocol with target ranges is provided with the kit. If control values are not within the limits of the target range, the assay results should be considered questionable and the samples should be tested again.

Observed Values

Observed values for intact proinsulin in EDTA plasma with the Human Intact Proinsulin ELISA kit were obtained from a group of healthy non-diabetic men and women.

Fasting values: ≤ 11 pmol/l

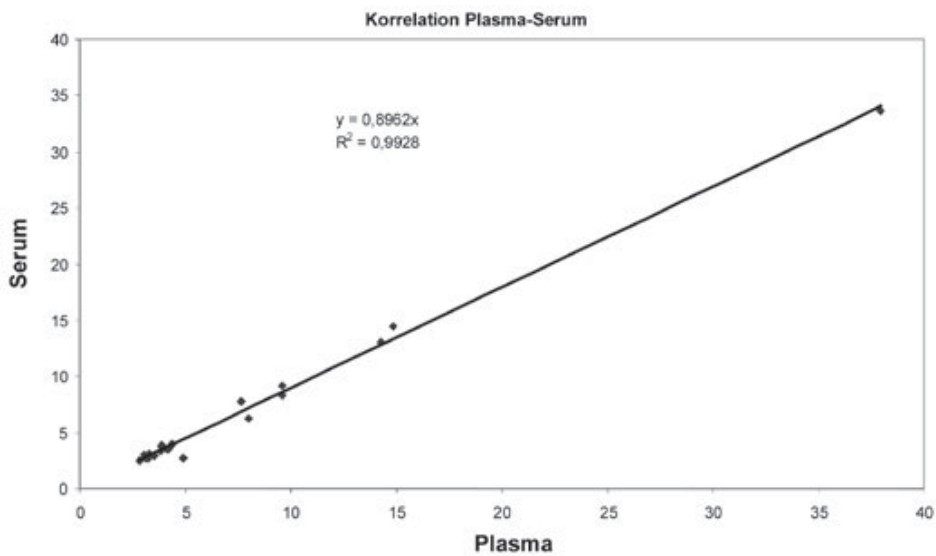
Mean value: 3.99 ± 1.58 SD, Median 3.61



Serum - EDTA Plasma

A regression analysis for plasma/serum gave the following results:

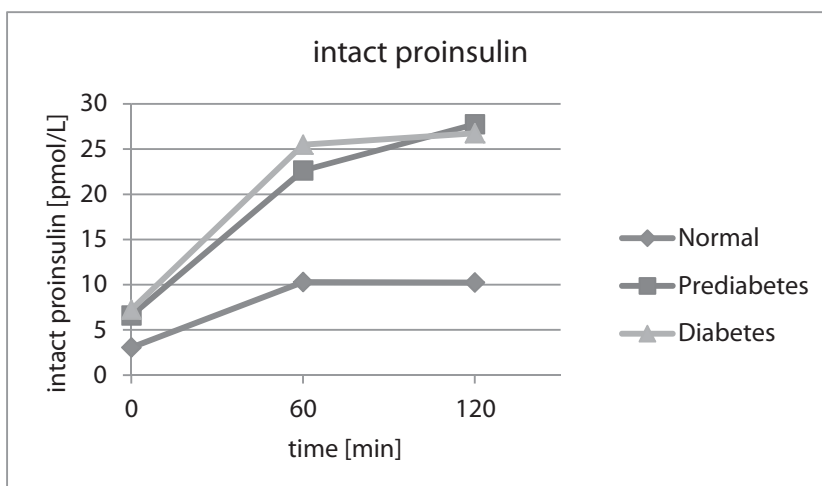
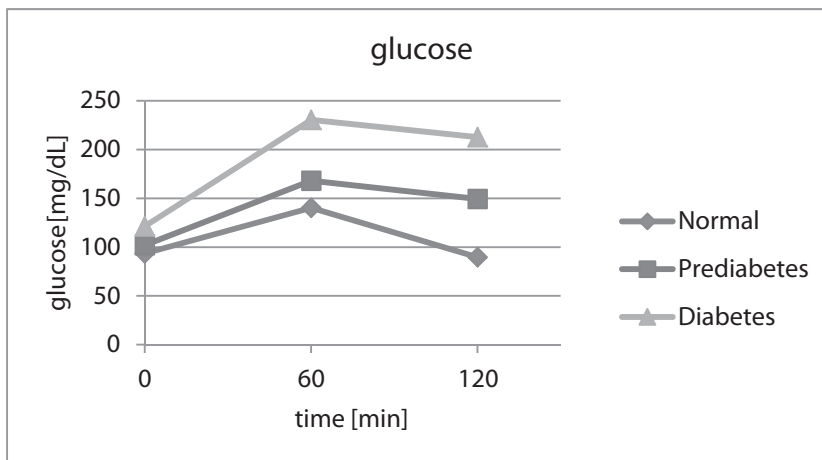
Y (Serum) = $0.89 \times X$ (Plasma) with $r = 0.99$ ($n = 20$)



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 (Pfützner et al., Diabetes Care 2004). In the majority of the cases, severe β -cell dysfunction with increased intact proinsulin secretion is preceding the final clinical onset of the disease with elevated glucose levels. This situation becomes most apparent during a glucose challenge test. As can be seen from the results, the secretion pattern of intact proinsulin 1 h and 2 h after start of the challenge is almost similar for patients in the prediabetic state and patients with overt type 2 diabetes mellitus.

Measuring intact proinsulin also during the glucose challenge at 0, 1 and 2 hours, can therefore be recommended as an indicator of β -cell functionality. It can be used to demonstrate the ongoing disease progression in patients with diabetes development even prior to disease manifestation. Healthy subjects will have values < 20 pmol/L at all timepoints. A value > 20 pmol/L at any timepoint is indicative for β -cell dysfunction and progressive disease development. Because of the multiple existing phenotypes of type 2 diabetes, a value < 20 pmol/L, however, does not automatically exclude diabetes in an individual patient.



Healthy individuals

Glucose

Time after glucose feeding (minutes)	0	60	120
Mean (mg/dL)	93.6	140.2	89.7
SD	7.5	29.4	23.9
Range (mg/dL)	81–104	104.4–178	67–140

Intact Proinsulin

Time after glucose feeding (minutes)	0	60	120
Mean (mg/dL)	3.1	10.3	10.2
SD	1.9	6.8	5.2
Range (mg/dL)	0.401–7.609	1.958–19.68	4.135–17.79

Prediabetes

Glucose

Time after glucose feeding (minutes)	0	60	120
Mean (mg/dL)	102.0	168.1	149.4
SD	9.3	56.9	33.8
Range (mg/dL)	92–122	74–243	93.6–186

Intact Proinsulin

Time after glucose feeding (minutes)	0	60	120
Mean (mg/dL)	6.6	22.6	27.8
SD	4.1	8.1	5.9
Range (mg/dL)	1.82–13.65	10.15–39.27	21.23–35.90

Diabetics

Glucose

Time after glucose feeding (minutes)	0	60	120
Mean (mg/dL)	121.3	230.4	212.7
SD	19.8	50.8	34.1
Range (mg/dL)	97–162	164–320	181–290

Intact Proinsulin

Time after glucose feeding (minutes)	0	60	120
Mean (mg/dL)	7.3	25.5	26.7
SD	7.0	8.8	10.1
Range (mg/dL)	2.47–25.14	5.54–39.43	3.94–43.81

Test Performance

Standard

This test is standardised against the 1. International Standard for Intact Proinsulin (WHO, IRR 84/611), 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 0.3 pmol/l.

Recovery Test

Serum Sample	Proinsulin Added pmol/l	Expected pmol/l	Observed pmol/l	Recovery (%)
Serum 1	0	3.98	3.98	100.00
	10	13.98	14.80	109.40
Serum 2	0	13.34	13.34	100.00
	10	23.34	23.32	106.20
Serum 3	0	3.68	3.68	100.00
	10	13.68	14.19	107.00
Serum 4	0	4.62	4.62	100.00
	10	14.62	12.53	88.80
Serum 5	0	5.01	5.01	100.00
	10	15.01	15.37	106.30

Plasma Sample	Proinsulin Added pmol/l	Expected pmol/l	Observed pmol/l	Recovery (%)
Plasma 1	0	51.10	51.10	100.00
	10	61.10	62.05	110.90
Plasma 2	0	54.13	54.13	100.00
	10	64.13	63.24	107.80
Plasma 3	0	47.15	47.15	100.00
	10	57.15	56.14	107.20
Plasma 4	0	36.85	36.85	100.00
	10	46.85	46.66	108.20
Plasma 5	0	38.38	38.38	100.00
	10	48.38	47.74	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

Plasma 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

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 specimens cannot be guaranteed.

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 ranges for Intact Proinsulin levels.

TECO® human Intakt Proinsulin

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature.
- Reconstitute the 2 vials Proinsulin 0 Standard **A** with 3 ml distilled water 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 lyophilized Standards **B** thru **F** and controls **L** and **H** with 1 ml distilled water each.

Prepare the required number of Assay Strips **1**

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

Pipette **50 µl** Standards **A** thru **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** thru **E**

Measure the absorbance at 405 nm, Standard **A** thru **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.