TECOmedical Group

TECO® *Proinsulin*

Human Intact Proinsulin ELISA

Instructions for Use
English

For Research Use Only.
Not for use in diagnostic procedures.

Catalogue No. TE1011

Symbol Description

Kit Instructions

Lot Number

Expiry Date

Storage Temperature

Manufacturer

Research Use Only

TE 1011

Attention

Intended use

Tests

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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	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
G	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
	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 human Proinsulin in plasma and serum.

Background

Proinsulin is produced in the pancreatic ß-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

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 zukünftigenTyp 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 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 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 antibodyis 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 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 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 research use only. Not for diagnostic procedures.
2 Treat all specimen samples as potentially biohazardous material. Follow General Precautions when handling contents of this kit and any 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, lyophilised. 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.
Preservative: Merthiolate. Store lyophilised at 2–8 °C until expiration date.

B TO F Standards
5 vials of lyophilised 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 standardised against the WHO International Reference reagent 09/296. Preservative: Merthiolate. Store lyophilised at 2–8 °C until expiration date.

L Control 1
1 vial of lyophilised 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 lyophilised at 2–8 °C until expiration date.

H Control 2
1 vial of lyophilised 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 lyophilised 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). 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
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. 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 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 E (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.

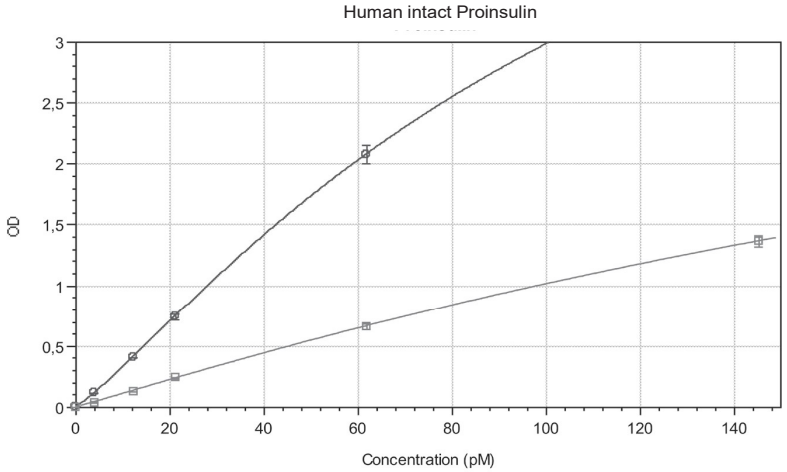
Result Analysis

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 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



Observed Values*

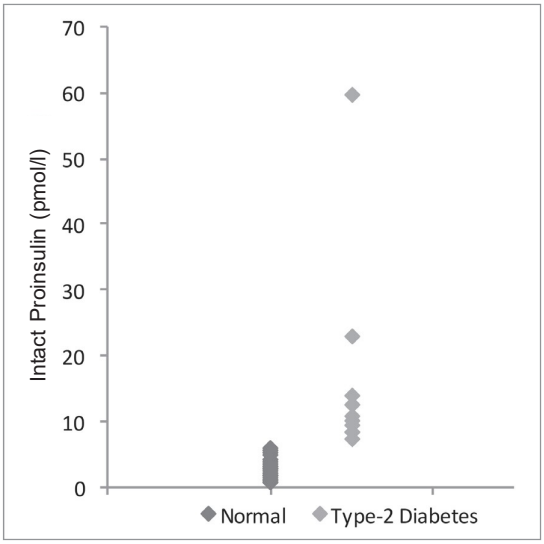
Normal values (N=32) for intact proinsulin in EDTA plasma were obtained with the Human Intact Proinsulin ELISA kit from a group of healthy men and women.

- Mean value: 2.67 ± 1.54 SD pmol/l

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

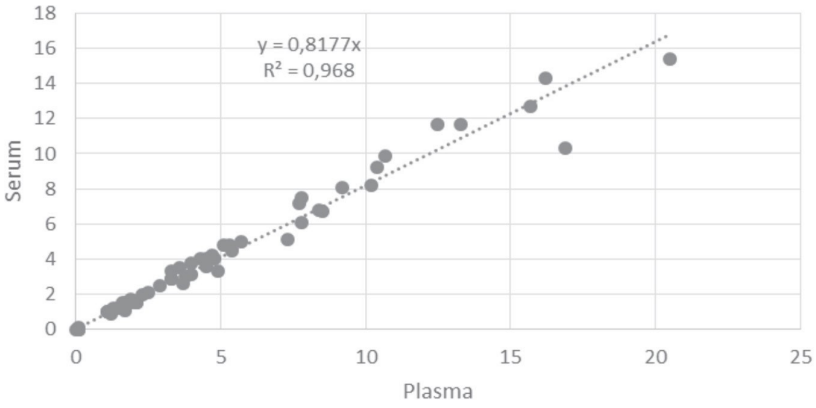
Fasting values:

- 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.



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

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	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

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

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

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 subject specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and subject history should be interpreted with caution. Samples from LADA subjects 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.