

Mercodia Proinsulin ELISA

Directions for Use
Mode d'emploi
Istruzioni per l'uso
Bruksanvisning

Gebrauchsinformation
Instrucciones para el uso
Brugsanvisning

10-1118-01
REAGENTS FOR 96 DETERMINATIONS






Manufactured by/Hersteller/Fabriqué par/
Fabricado por/Prodotto da/Fremstillet af/
Tillverkad av

Mercodia AB, Sylveniusgatan 8A,
SE-754 50 Uppsala,
Sweden, Schweden, Suède, Suecia, Svezia, Sverige

Mercodia 

The Mercodia logo is a stylized grey triangle pointing to the right, with a small white circle inside it.

EXPLANATION OF SYMBOLS USED ON LABELS/ERKLÄRUNG DER SYMBOLE AUF DEN ETIKETTEN/EXPLICATION DES SYMBOLES UTILISES SUR LES ETIQUETTES/EXPLICACIÓN DE LOS SÍMBOLOS UTILIZADOS EN LAS ETIQUETAS/SPIEGAZIONE DEI SIMBOLI USATI SULLE ETICHETTE/FORKLARING AF SYMBOLER ANVENDT PÅ ETIKETTER/FÖRKLARING AV SYMBOLERNA SOM ANVÄNDS PÅ ETIKETTERNA

 <p>$\Sigma = 96$</p>	<p>Reagents for 96 determinations Reagenzien für 96 Bestimmungen Réactifs pour 96 mesures Reactivos para 96 determinaciones Reagenti per 96 rilevazioni Reagens til 96 bestemmelser Reagenser för 96 bestämningar</p>
	<p>Expiry date Verfallsdatum A utiliser avant Fecha de caducidad Data di scadenza Udløbsdato Utgångsdatum</p>
	<p>Store between 2–8°C Lagerungstemperatur 2–8°C A conserver entre 2 et 8 °C Conservar a entre 2–8 °C Conservare tra i 2–8 °C Opbevar ved 2–8°C Förvara vid 2–8°C</p>
<div style="border: 1px solid black; padding: 5px; text-align: center; width: fit-content; margin: auto;">LOT</div>	<p>Lot No. Lot Nr. N° de lot N° lote Lotto n. Partinr. Lotnr.</p>
<div style="border: 1px solid black; padding: 5px; text-align: center; width: fit-content; margin: auto;">IVD</div>	<p>For <i>in vitro</i> diagnostic use Zum Gebrauch in der <i>in vitro</i>-Diagnose Ce kit est réservé à l'utilisation diagnostique <i>in vitro</i> Para uso diagnóstico <i>in vitro</i> Per l'uso diagnostico <i>in vitro</i> Til <i>in vitro</i>-diagnosticering För <i>in vitro</i> diagnostiskt bruk</p>

INTENDED USE

Mercodia Proinsulin ELISA provides a method for the quantitative determination of human proinsulin in serum or plasma .

SUMMARY AND EXPLANATION OF THE TEST

Proinsulin is the precursor of insulin which is the principle hormone responsible for the control of glucose metabolism. It is synthesized in the β -cells of the Islets of Langerhans and is subsequently processed to form C-peptide and insulin. High proinsulin concentrations are usually noted in patients with benign or malignant β -cell tumors of the pancreas. Most patients with β -cell tumors have increased insulin, C-peptide and proinsulin concentrations, but occasionally only proinsulin is elevated. Despite its low biological activity, proinsulin may be increased sufficiently to produce hypoglycemia. Increased proinsulin concentrations may also be detected in patients with renal failure, cirrhosis, or hyperthyroidism.

PRINCIPLE OF THE PROCEDURE

Mercodia Proinsulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the proinsulin molecule. During incubation, proinsulin in the sample reacts with anti-proinsulin antibodies bound to microtitration well. After washing, peroxidase-conjugated anti-proinsulin antibodies are added and after the second incubation and a simple washing step that removes unbound enzyme labeled antibody, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB) The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5 M H_2SO_4 . Follow routine precautions for handling hazardous chemicals.
- All patient specimens should be handled as if capable of transmitting infections.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 50, 100, 200 and 1000 μ l (repeat pipettes preferred for addition of Enzyme Conjugate, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Microplate reader (450 nm filter)
- Plate shaker (The recommended velocity is 700–900 cycles per minute, orbital movement)
- Microplate washing device

REAGENTS

Each Mercodia Proinsulin ELISA kit contains reagents for 96 wells, sufficient for 42 samples and one calibration curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical Lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is +2–8°C.

Coated Plate (mouse monoclonal anti-proinsulin) 8-well strips. For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.	1 plate	96 wells	Ready for use
Calibrators Concentration indicated on vial label. Use within 4 weeks. For storage longer than 4 weeks, store at –20°C.	4 vials	1000 μ l	Lyophilized Add 1000 μ l redistilled water per vial.
Calibrator 0 Color coded yellow	1 vial	5 ml	Ready for use
Assay Buffer Color coded red	1 vial	6 ml	Ready for use
Enzyme Conjugate 21X (Peroxidase conjugated mouse monoclonal anti-proinsulin)	1 vial	600 μ l	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	12 ml	Ready for use
Wash Buffer 21X Dilute with 800 ml redistilled water to make Wash Buffer. Storage after dilution: +2–8°C for 4 weeks	1 bottle	40 ml	
Substrate TMB (TMB) Colorless solution. <i>Note! Light sensitive!</i>	1 vial	22 ml	Ready for use
Stop Solution 0.5 M H ₂ SO ₄	1 vial	7 ml	Ready for use

Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate solution by dilution of the Enzyme Conjugate 21X in Enzyme Conjugate Buffer according to the table below.

When preparing enzyme conjugate solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 21X vial. Mix gently before use.

Number of strips	Enzyme Conjugate 21X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	350 µl	7.0 ml
6 strips	250 µl	5.0 ml
4 strips	200 µl	4.0 ml

Storage after dilution: 2-8°C for two months.

SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Samples can be stored at 2–8°C up to 24 hours. For longer periods store samples at –20°C. Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing heparin or EDTA as anticoagulant, and separate the plasma fraction. Samples can be stored at 2–8°C up to 24 hours. For longer periods store samples at –20°C. Avoid repeated freezing and thawing.

Preparation of samples

No dilution is normally required, however, samples containing >132 pmol/l should be diluted 1/10 v/v with Calibrator 0.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Prepare a calibration curve for each assay run.

1. Prepare sufficient microplate wells to accommodate calibrators and samples in duplicate
2. Pipette 50 μ l each of calibrators and samples into appropriate wells.
3. Add 50 μ l Assay Buffer to each well.
4. Incubate on a plate shaker for 1 hour at room temperature (18–25°C)
5. Wash 6 times with automatic washer or:
Aspirate the reaction volume and fill each well completely with 350 μ l Wash Buffer. Discard liquid completely. Repeat 5 times.
After final wash, invert and tap the plate firmly against absorbent paper.
6. Add 100 μ l enzyme conjugate solution to each well
7. Incubate on a plate shaker for 1 hour at room temperature. (18–25°C)
8. Wash as described above
After final wash, invert and tap the plate firmly against absorbent paper.
9. Add 200 μ l Substrate TMB
10. Incubate for 15 minutes at room temperature (18–25°C).
11. Add 50 μ l Stop Solution.
Place plate on a shaker for approximately 5 seconds to ensure mixing.
12. Read optical density at 450 nm and calculate results
Read within 30 minutes.

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

INTERNAL QUALITY CONTROL

Commercial controls such as Mercodia Diabetes antigen control (Code No. 10-1164-01/10-1134-01) and/or internal serum pools with low, intermediate and high proinsulin concentrations should routinely be assayed as unknowns, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number; reconstitution dates of kit components; OD values for the blank, Calibrators and controls.

CALCULATION OF RESULTS

Computerized calculation

The concentration of proinsulin is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the concentration using cubic spline regression.

Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the proinsulin concentration on a log-log paper and construct a calibration curve.
2. Read the concentration of the unknown samples from the calibration curve.

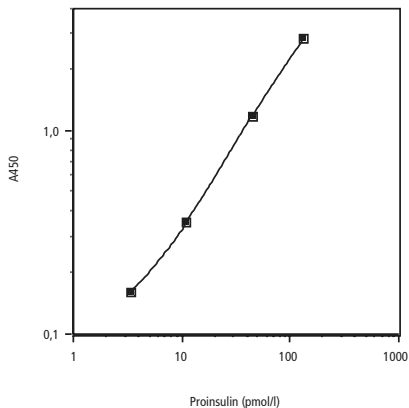
Example of results

Wells	Identity	A ₄₅₀	Mean conc. pmol/l
1A–B	Calibrator 0	0.070/0.069	
1C–D	Calibrator 3.3 pmol/l *	0.156/0.163	
1E–F	Calibrator 11 pmol/l *	0.347/0.354	
1G–H	Calibrator 44 pmol/l *	1.157/1.176	
2A–B	Calibrator 132 pmol/l *	2.862/2.831	
2C–D	Unknown 1	0.208/0.208	5.25
2E–F	Unknown 2	0.252/0.254	7.07
2G–H	Unknown 3	0.563/0.589	19.8
3A–B	Unknown 4	1.592/1.571	63.2

*Concentration indicated on vial label.

Example of calibration curve

A typical calibration curve is shown here. Do not use this curve to determine actual assay results.



LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.

Application of this test to individuals already undergoing insulin therapy is complicated by formation of anti-insulin antibodies that are capable of interfering in the assay.

Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own. Fasting levels for 112 tested, apparently healthy individuals, yielded a mean of 10 pmol/l, a median of 7 pmol/l and a range- corresponding to the central 95 % of the observations- of 3.3–28 pmol/l.

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit is 0.5 pmol/l calculated as two standard deviations above the Calibrator 0.

Recovery

Recovery upon addition is 97 %

Hook effect

Samples with a concentration of up to 80 000 pmol/l can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4-replicates on seven different occasions.

Sample	Mean value pmol/l	Coefficient of variation		
		within assay %	between assay %	total assay %
1	7.3	3.2	3.9	5.1
2	20.7	3.2	5.2	6.1
3	65.6	2.5	4.2	5.0

Specificity

The following cross reactions have been found:

Insulin	<0.03 %
C-peptide	<0.006 %
Proinsulin Des(64–65)	84 %
Proinsulin Split (65–66)	90 %
Proinsulin Des(31–32)	95 %
Proinsulin Split (32–33)	95 %

CALIBRATION

Mercodia Proinsulin ELISA kit is calibrated against the International Reference Reagent for human proinsulin, IRR 84/611.

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Merckodia AB may affect the results, in which event Merckodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

REFERENCES

- Gaines-Das, RE and Bristiw AF: WHO International reference reagents for human proinsulin and human insulin C-peptide. *J Biol Stand* 16:179-186, 1988
- Garcia CA, Prabakar KR, Diez J, Cao ZA, Allende G, Zeller M, Dogra R, Mendez A, Rosenkranz E, Dahl U, Ricordi C, Hanahan D, Pugliese A. Dendritic cells in human thymus and periphery display a proinsulin epitope in a transcription-dependent, capture-independent fashion. *J Immunol.* 2005 Aug 15;175(4):2111-22.
- Riserus U, Vessby B, Arner P, Zethelius B. Supplementation with trans10cis12-conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity. *Diabetologia.* 2004 Jun;47(6):1016-9.
- Yin H, Berg AK, Westman J, Hellerstrom C, Frisk G. Complete nucleotide sequence of a Coxsackievirus B-4 strain capable of establishing persistent infection in human pancreatic islet cells: effects on insulin release, proinsulin synthesis, and cell morphology. *J Med Virol.* 2002 Dec;68(4):544-57.

**SUMMARY PROTOCOL SHEET/ZUSAMMENFASSUNG DES PROTOKOLLBLATTES/
FEUILLE DE PROTOCOLE RESUMEE/HOJA DE RESUMEN DEL PROTOCOLO/PRO-
TOCOLLO DI SINTESI/OVERSIGTS-PROTOKOLARK/SAMMANFATTNINGS-PROTOKOLL**

Mercodia Proinsulin ELISA

Add Calibrators and samples 50 µl Kalibrator und Proben begeben Ajout de Calibrators et d'échantillons Añadir Calibrators y muestras Aggiungere Calibrators e campioni Tilsæt Calibrators og prøver Tillsätt Calibrators och prover	Wash 6 times Waschen 6 mal Rinçage 6 rinçages Lavar 6 veces Lavare 6 volte Skyl 6 gange Tvätta 6 gånger
Add Assay Buffer 50 µl Assay Buffer beifügen Ajout d'Assay Buffer Añadir Assay Buffer Aggiungere Assay Buffer Tilsæt Assay Buffer Tillsätt Assay Buffer	Add Substrate TMB 200 µl TMB Substrat begeben Ajout de Substrat (TMB) Añadir Substrate TMB Aggiungere Substrate TMB Tilsæt Substrate-TMB Tillsätt Substrate TMB
Incubate 1 hour at 18–25°C on a shaker Inkubieren 1 Stunde auf einem Schüttler bei 18–25°C Incubation 1 heure à 18–25°C sur un agitateur seoueur de plaques Incubar 1 hora a 18–25 °C en un agitador de placas Incubazione 1 ora a 18–25° C in una piastra shaker Inkuber 1 time ved 18–25°C på et ryste-bord Inkubera 1 timme vid 18–25°C på en plattskak	Incubate 15 minutes Inkubieren 15 Minuten Incubation 15 minutes Incubar 15 min Incubazione 15 minuti Inkuber 15 min Inkubera 15 minuter
Wash 6 times Waschen 6 mal Rinçage 6 rinçages Lavar 6 veces Lavare 6 volte Skyl 6 gange Tvätta 6 gånger	Add Stop Solution 50 µl Shake for 5 sec to ensure mixing Stopplösung beifügen 50 µl Sicherstellen von Durchmischung 5 Sek. schütteln Ajout de Stop Solution 50 µl Secouer pendant 5 secondes pour bien mélanger Añadir Stop Solution 50 µl Agitar durante 5 segundos para asegurar el mezclado Aggiungere Stop Solution 50 µl Scuotere per 5 secondi per assi curarsi che sia tutto mescolato Tilsæt Stop Solution 50 µl Ryst i 5 sekunder for sikre blanding Tillsätt Stop Solution 50 µl Skaka i 5 sekunder för att se till att lösningen blandas
Add enzyme conjugate solution 100 µl Enzymkonjugatlösung beifügen Ajout du conjugué enzymatique Añadir la disolución de conjugado enzimático Aggiungere la soluzione enzima coniugato Tilsæt enzymkonjugateopløsning Tillsätt enzymkonjugatlösning	Measure A ₄₅₀ Messung A ₄₅₀ Mesure de A ₄₅₀ Medir A ₄₅₀ Misura A 450 Af læs A ₄₅₀ Mät vid A ₄₅₀
Incubate 1 hour at 18–25°C on a shaker Inkubieren 1 Stunde auf einem Schüttler bei 18–25°C Incubation 1 heure à 18–25°C sur un agitateur seoueur de plaques Incubar 1 hora a 18–25 °C en un agitador de placas Incubazione 1 ora a 18–25° C in una piastra shaker Inkuber 1 time ved 18–25°C på et ryste-bord Inkubera 1 timme vid 18–25°C på en plattskak	