

Mercodia Iso-Insulin ELISA

Directions for Use

Mode d'emploi

Istruzioni per l'uso

Bruksanvisning

Gebrauchsinformation

Instrucciones para el uso

Brugsanvisning

10-1128-01

REAGENTS FOR 96 DETERMINATIONS








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

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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 UTILISÉS SUR LES ÉTIQUETTES/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>
	<p>Lot no Lot Nr. N° de lot N° lote Lotto n. Partinr. Lotnr.</p>
	<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 Iso-Insulin ELISA provides a method for the quantitative determination of insulin in human serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesised in the β -cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain.

Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. Its principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, epinephrine (adrenaline), growth hormone and cortisol.

Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

PRINCIPLE OF THE PROCEDURE

Mercodia Iso-Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample react with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microtitration well. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine. 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 contents 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 25, 50 and 200 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 with 450 nm filter
- Plate shaker (The recommended velocity is 700–900 cycles per minute, orbital movement)
- Microplate washing device

REAGENTS

Each Mercodia Iso-Insulin ELISA kit contains reagents for 96 wells, sufficient for 43 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-insulin)	1 plate 8-well strips	96 wells	Ready for use
For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.			
Calibrators 3; 10; 30 and 100 mU/l (recombinant human insulin)	4 vials	1000 μ l	Ready for use
Calibrator 0 Color coded yellow	1 vial	5 ml	Ready for use
Enzyme Conjugate 11X (peroxidase conjugated mouse monoclonal anti-insulin)	1 vial	600 μ l	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	6 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 by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer or according to the table below. Mix gently.

When preparing enzyme conjugate solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	350 µl	3.5 ml
6 strips	250 µl	2.5 ml
4 strips	200 µl	2.0 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

Samples containing >100 mU/l should be diluted e.g. 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 25 μ l each of Calibrators and samples into appropriate wells.
- 3 Pipette 50 μ l enzyme conjugate solution 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 200 μ l Substrate TMB.
- 7 Incubate for 15 minutes at room temperature (18–25°C).
- 8 Add 50 μ l Stop Solution.
Place plate on a shaker for approximately 5 seconds to ensure mixing.
- 9 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 10-1134-01/10-1164-01) and/or internal serum pools with low, intermediate and high insulin 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 insulin 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

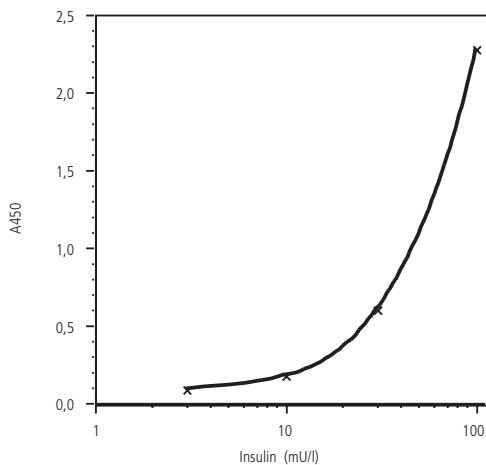
1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the insulin concentration on a lin-log paper and construct a calibration curve.
2. Read the concentration of the unknown samples from the calibration curve.

Example of results

Wells	Identity	A ₄₅₀	Mean conc. mU/l
1A–B	Calibrator 0	0.066/0.067	
1C–D	Calibrator 3 mU/l	0.084/0.087	
1E–F	Calibrator 10 mU/l	0.161/0.165	
1G–H	Calibrator 30 mU/l	0.595/0.599	
2A–B	Calibrator 100 mU/l	2.377/2.347	
2C–D	Unknown 1	0.270/0.272	16.4
2E–F	Unknown 2	1.146/1.192	53.6
2G–H	Unknown 3	2.044/2.150	92.7

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.

Comparison studies between Mercodia Iso-Insulin ELISA and Mercodia Insulin ELISA, have been performed with 90 samples. The values found show a good correlation between the two techniques, $r=0.98$.

Thus, the expected values for Mercodia Insulin ELISA can be used for Mercodia Iso-Insulin ELISA as well.

Mean fasting levels for 137 tested, apparently healthy individuals, were 10 mU/l, a median of 7 mU/l and a range- corresponding to the central 95% of the observations – of 2–25 mU/l.

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit is 1 mU/l calculated as two standard deviations above the Calibrator 0.

Recovery

Recovery upon addition is 101%.

Hook effect

Samples with a concentration of up to at least 2000 mU/l can be measured without giving falsely low results.

Precision

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

Sample	Mean value mU/l	Coefficient of variation		
		within assay %	between assay %	total assay %
1	15.9	3.0	3.9	4.9
2	53.2	2.8	3.0	4.1
3	90.9	3.2	3.0	4.4

Specificity

insulin	100%
Insulin lispro (Humalog®, Eli Lilly)	100%
Insulin aspart	100%
C-peptide	< 0.1%
Proinsulin	54%
Proinsulin des (31-32)	58%
Proinsulin split (32-33)	56%
Proinsulin des (64-65)	66%
Proinsulin split (65-66)	78%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	71%
Mouse insulin	49%
Porcine insulin	306%
Sheep insulin	131%
Bovine insulin	58%

CALIBRATION

Mercodia Iso-Insulin ELISA kit is calibrated against 1st International Reference Preparation 66/304 for human insulin.

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia 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.

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Hedman CA, Lindstrom T, Arnqvist HJ: Direct comparison of insulin lispro and aspart shows small differences in plasma insulin profiles after subcutaneous injection in type 1 diabetes. *Diabetes Care* 24:1120-1121, 2001

Lindstrom T, Hedman CA, Arnqvist HJ: Use of a novel double-antibody technique to describe the pharmacokinetics of rapid-acting insulin analogs. *Diabetes Care* 25: 1049-1054, 2002

Heise T, Nosek L, Biilmann Ronn B, Endahl L, Heinemann L, Kapitza C, Draeger E: Lower Within-Subject Variability of Insulin Detemir in Comparison to NPH Insulin and Insulin Glargine in People With Type 1 Diabetes. *Diabetes* 53:1614-1620, 2004

**SUMMARY PROTOCOL SHEET/ZUSAMMENFASSUNG DES PROTOKOLLBLATTES/
FEUILLE DE PROTOCOLE RESUMEE/HOJA DE RESUMEN DEL PROTOCOLO/PRO-
TOCOLLO DI SINTESI/OVERSIGTSPROTOKOLARK/SAMMANFATTNINGSPROTOKOLL**

Merckodia Iso-Insulin ELISA

<p>Add Calibrators and samples 25 µl Kalibrator och Proben begeben Ajout de Calibrators et d'échantillons Añadir Calibrators y muestras Aggiungere Calibrators e campioni Tilsæt Calibrators og prøver Tillsätt Calibrators och prover</p>	<p>Incubate 15 minutes (18–25°C) Inkubieren 15 Minuten (18–25°C) Incubation 15 minutes (18–25°C) Incubar 15 min (18–25°C) Incubazione 15 minuti (18–25°C) Inkuber 15 min (18–25°C) Inkubera 15 minuter (18–25°C)</p>
<p>Add enzyme conjugate solution 50 µl Enzymkonjugatlösung beifügen Ajout du conjugué enzymatique Añadir disolución conjugado enzimático Aggiungere soluzione enzima coniugato Tilsæt enzymkonjugatopløsning Tillsätt enzymkonjugatlösning</p>	<p>Add Stop Solution 50 µl Shake for 5 sec to ensure mixing Stoplösung 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 assicurarsi 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</p>
<p>Incubate 1 hour at 18–25°C on a shaker Inkubieren 1 Stunde auf einem Schüttler bei 18–28°C Incubation 1 heure à 18–25°C sur un agitateur secouer 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 rystebord Inkubera 1 timme vid 18–25°C på en plattskak</p>	<p>Measure A₄₅₀ Messung A₄₅₀ Mesure de A₄₅₀ Medir A₄₅₀ Misura A₄₅₀ Aflæs A₄₅₀ Mät vid A₄₅₀</p>
<p>Wash 6 times Waschen 6 mal Rinçage 6 rinçages Lavar 6 veces Lavare 6 volte Skyl 6 gange Tvätta 6 gånger</p>	
<p>Add Substrate TMB 200 µl TMB Substrat begeben Ajout de Substrat (TMB) Añadir Substrate TMB Aggiungere Substrate TMB Tilsæt Substrate-TMB Tillsätt Substrate TMB</p>	