

Mercodia Insulin ELISA

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

Gebrauchsinformation
Instrucciones para el uso
Brugsanvisning

10-1113-01

Reagents for 96 determinations

10-1113-10

Reagents for 10 x 96 determinations






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

The Mercodia logo is a stylized grey arrow pointing to the right, with a small circle at its base.

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; display: inline-block;"> <p>LOT</p> </div>	<p>Lot No. Lot Nr. N° de lot N° lote Lotto n. Partinr. Lotnr.</p>
<div style="border: 1px solid black; padding: 5px; display: inline-block;"> <p>IVD</p> </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 Insulin ELISA provides a method for the quantitative determination of human insulin in serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized 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 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 reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microtitration well. A simple washing step removes unbound enzyme labelled 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 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, 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 1 X 96

Each Mercodia Insulin ELISA kit (10-1113-01) contains reagents for 96 wells, sufficient for 42 samples and one calibrator 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) 8-well strips.	1 plate	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, 100 and 200 mU/l (recombinant human insulin)	5 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	1.2 ml	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:	1 bottle	40 ml	+2–8°C for 4 weeks.
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 Enzyme Conjugate 11X, (1+10) 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 11X vial. Mix gently. Use within one day.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µl	7.0 ml
6 strips	500 µl	5.0 ml
4 strips	350 µl	3.5 ml

REAGENTS 10 X 96

Each Mercodia Insulin ELISA kit (10-1113-10) contains reagents for 10 × 96 wells, sufficient for 42 samples and one calibrator curve in duplicate on each plate. 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) 8-well strips	10 plates	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; 100 and 200 mU/l (recombinant human insulin)	5 vials	1000 µl	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	12 ml	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 bottle	120 ml	Ready for use
Wash Buffer 21X	2 bottle	200 ml	Preparation, see below
Substrate TMB (TMB) Colorless solution. <i>Note! Light sensitive!</i>	1 bottle	220 ml	Ready for use
Stop Solution 0.5 M H ₂ SO ₄	1 bottle	70 ml	Ready for use

Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate solution by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer according to the table below. Mix gently. Use within one day.

Number of plates	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
10 plates	1 vial	1 bottle
5 plates	5.0 ml	50 ml
3 plates	3.0 ml	30 ml
2 plates	2.0 ml	20 ml
1 plate	1.0 ml	10 ml

Preparation of wash buffer

Prepare the needed volume of wash buffer by dilution of Wash Buffer 21X in redistilled water (1+20) according to the table below. Mix properly.

Number of plates	Wash Buffer 21X	Redistilled water
10 plates	2 bottles	8000 ml
5 plates	180 ml	3600 ml
3 plates	110 ml	2200 ml
2 plates	70 ml	1400 ml
1 plate	35 ml	700 ml

Storage after dilution: +2–8°C for 4 weeks.

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 >200 m U/l should be diluted 1+9 v/v with Calibrator 0.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use.

Prepare a calibrator curve for each assay run.

1. Prepare enzyme conjugate solution and wash buffer.
2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
3. Pipette 25 μ l each of Calibrators and samples into appropriate wells.
4. Add 100 μ l of enzyme conjugate solution to each well.
5. Incubate on a plate shaker for 1 hour at room temperature (18–25°C)
6. Wash plate 6 times with automatic plate washer
or
Aspirate the reaction volume. Add 350 μ l wash buffer to each well. Aspirate completely. Repeat 5 times.
After final wash, invert and tap the plate firmly against absorbent paper.
7. Add 200 μ l Substrate TMB into each well
8. Incubate for 15 minutes at room temperature (18–25°C)
9. Add 50 μ l Stop Solution to each well.
Place plate on a shaker for approximately 5 seconds to ensure mixing.
10. 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 concentration 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 date of 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

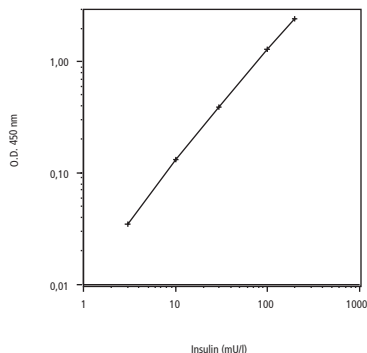
1. Plot the absorbance values obtained for the Calibrators, except for Calibrator 0, against the insulin concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.

Example of results

Wells	Identity	A ₄₅₀	Mean conc. mU/l
1 A-B	Calibrator 0	0.070/0.071	
1 C-D	Calibrator 3 mU/l	0.105/0.106	
1 E-F	Calibrator 10 mU/l	0.202/0.204	
1 G-H	Calibrator 30 mU/l	0.434/0.470	
2 A-B	Calibrator 100 mU/l	1.348/1.351	
2 C-D	Calibrator 200 mU/l	2.451/2.476	
2 E-F	Unknown 1	0.222/0.214	11.1
2 G-H	Unknown 2	0.546/0.538	35.6
3 A-B	Unknown 3	1.941/1.978	153

Example of calibrator curve

A typical calibrator 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 hemolysed 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 137 tested, apparently healthy individuals, yielded a mean of 9.2 mU/l, a median of 6.9 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 94–113% (mean 104%)

Hook effect

Samples with a concentration of up to 30 000 mU/l can be measured without giving falsely low results.

Precision

Each sample was analysed in 6-replicates on six different occasions.

Sample	Mean value mU/l	Coefficient of variation		
		within assay %	between assay %	total assay %
1	11	3.4	3.6	5.0
2	36	4.0	2.6	4.7
3	80	2.8	2.8	4.0
4	154	3.2	2.9	4.4

Specificity

The following crossreactions have been found:

C-peptide	< 0.01%
Proinsulin	< 0.01%
Proinsulin des (31-32)	< 0.5%
Proinsulin split (32-33)	< 0.5%
Proinsulin des (64-65)	98%
Proinsulin split (65-66)	56%
Insulin lispro (Humalog®, Eli Lilly)	< 0.006%
Insulin aspart	< 0.006%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	0.7%
Mouse insulin	0.3%
Porcine insulin	374%
Sheep insulin	48%
Bovine insulin	31%

CALIBRATION

Mercodia Insulin ELISA kit is calibrated against 1st International Reference Preparation 66/304.

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 authorised distributors, in such event, shall not be liable for damages indirect or consequential.

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

Mercodia Insulin ELISA

<p>Add Calibrators and samples 25 µl Calibrators 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</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 100 µl Enzyme Conjugate beifügen Ajout d'Enzyme Conjugate Añadir Enzyme Conjugate Aggiungere Enzyme Conjugate Tilsæt Enzyme Conjugate Tillsätt Enzyme Conjugate</p>	<p>Add Stop Solution 50 µl Shake for 5 sec to ensure mixing Stop Solution beifügen 50 µl Sicherstellen von Durchmischung 5 Sek. schütteln Ajout de Stop Solution 50 µl Secouer pendant 5 secondes pour bien mélanger</p>
<p>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 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 ryste-bord Inkubera 1 timme vid 18–25°C på en plattskak</p>	<p>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</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>Measure A₄₅₀ Messung A₄₅₀ Mesure de A₄₅₀ Medir A₄₅₀ Misura A₄₅₀ Aflæs A₄₅₀ Mät vid A₄₅₀</p>
<p>Add Substrate TMB 200 µl Substrate TMB begeben Ajout de Substrate (TMB) Añadir Substrate TMB Aggiungere Substrate TMB Tilsæt Substrate-TMB Tillsätt Substrate TMB</p>	