

# Mercodia Ultrasensitive Insulin ELISA

Directions for Use

Mode d'emploi

Istruzioni per l'uso

Bruksanvisning

Gebrauchsinformation

Instrucciones para el uso

Brugsanvisning

**10-1132-01**

**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 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><math>\Sigma = 96</math></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><b>LOT</b></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><b>IVD</b></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 Ultrasensitive 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 synthesised in the  $\beta$ -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 utilisation 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 Ultrasensitive 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  $\mu$ 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 Ultrasensitive Insulin ELISA kit (10-1132-01) 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.

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<b>Coated Plate</b> (mouse monoclonal anti-insulin) 8-well strips	1 plate	96 wells	Ready for use
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For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.

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<b>Calibrators</b> 0.15; 1; 3; 10 and 20 mU/l (recombinant human insulin)	5 vials	1000 $\mu$ l	Ready for use
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<b>Calibrator 0</b> Colour coded yellow	1 vial	5 ml	Ready for use
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<b>Enzyme Conjugate 11X</b> (peroxidase conjugated mouse monoclonal anti-insulin)	1 vial	1.2 ml	Preparation, see below
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<b>Enzyme Conjugate Buffer</b> Colour coded blue	1 vial	12 ml	Ready for use
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<b>Wash Buffer 21X</b> Dilute with 800 ml redistilled water to make Wash Buffer Storage after dilution: +2–8°C for 4 weeks.	1 bottle	40 ml	
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<b>Substrate TMB</b> (TMB) Colourless solution <i>Note! Light sensitive!</i>	1 vial	22 ml	Ready for use
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<b>Stop Solution</b> 0.5 M H <sub>2</sub> SO <sub>4</sub>	1 vial	7 ml	Ready for use
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## Preparation of Enzyme Conjugate

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. Use within one day.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
4 strips	400 µl	4.0 ml
6 strips	500 µl	5.0 ml
8 strips	700 µl	7.0 ml
10 strips	900 µl	9.0 ml
12 strips	1 vial	1 vial

## 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 >20mU/l should be diluted 1/10v/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  $\mu$ l each of Calibrators and samples into appropriate wells.
3. Add 100  $\mu$ l of Enzyme Conjugate to each well.
4. Incubate on a plate shaker for 1 hour at room temperature (18–25°C)
5. Wash plate 6 times with automatic plate washer  
or  
Aspirate the reaction volume and fill each well completely with 350  $\mu$ l Wash Buffer. Discard liquid completely. Repeat 5 times.  
After final wash, invert and tap the plate firmly against absorbent paper.
6. Add 200  $\mu$ l Substrate TMB into each well
7. Incubate for 30 minutes at room temperature (18–25°C)
8. Add 50  $\mu$ l Stop Solution to each well.  
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

## 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

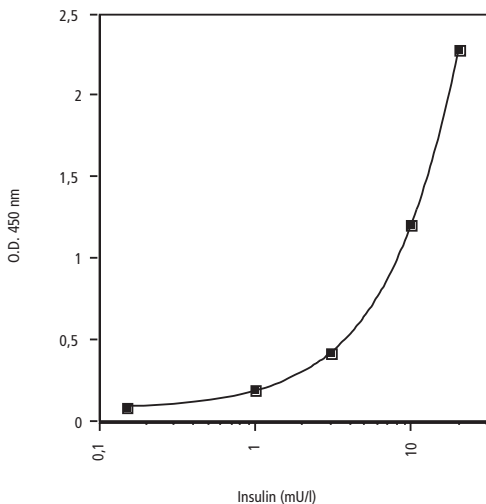
1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the insulin concentration on a lin-log paper and construct a calibration curve.
2. Read the concentration of the unknown samples from the calibration curve.

## Example of results

Mean Wells	Identity	A <sub>450</sub>	conc. mU/l
1A–B	Calibrator 0	0.071/0.073	
1C–D	Calibrator 0.15 mU/l	0.087/0.088	
1E–F	Calibrator 1.0 mU/l	0.186/0.188	
1G–H	Calibrator 3.0 mU/l	0.405/0.424	
2A–B	Calibrator 10 mU/l	1.172/1.241	
2C–D	Calibrator 20 mU/l	2.262/2.290	
2E–F	Unknown 1	0.466/0.470	3.5
2G–H	Unknown 2	1.987/1.926	17.0
3A–B	Unknown 3	0.327/0.317	2.2

## 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 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 0.07 mU/l calculated as two standard deviations above the Calibrator 0

### Recovery

Recovery upon addition is 97–107% (mean 102%)

### Hook effect

Samples with a concentration of up to 800 000 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	5.7	5.3	2.7	6.0
2	8.2	4.2	3.9	5.8
3	15.5	5.1	1.8	5.4

## 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 Ultrasensitive 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.

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

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	Incubate 30 minutes Inkubieren 30 Minuten Incubation 30 minutes Incubar 30 min Incubazione 30 minuti Inkuber 30 min Inkubera 30 minuter
Add Enzyme Conjugate 100 µl Enzyme Conjugate beifügen Ajout d'Enzyme Conjugate Añadir Enzyme Conjugate Aggiungere Enzyme Conjugate Tilsæt Enzyme Conjugate Tillsätt Enzyme Conjugate	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 Añadir Stop Solution 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
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 secoueur 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	Measure A <sub>450</sub> Messung A <sub>450</sub> Mesure de A <sub>450</sub> Medir A <sub>450</sub> Misura A <sub>450</sub> Aflæs A <sub>450</sub> Mät vid A <sub>450</sub>
Wash 6 times Waschen 6 mal Rinçage 6 rinçages Lavar 6 veces Lavare 6 volte Skyl 6 gange Tvätta 6 gånger	
Add Substrate TMB 200 µl Substrate TMB begeben Ajout de Substrat (TMB) Añadir Substrate TMB Aggiungere Substrate TMB Tilsæt Substrate-TMB Tillsätt Substrate TMB	