Mercodia
Developing diagnostics

# Mercodia Ultrasensitive Insulin ELISA

Directions for Use

**10-1132-01**
**REAGENTS FOR 96 DETERMINATIONS**

**IVD**

$C\epsilon$

Gebrauchanweisung in deutsch finden Sie under folgenden Link:
Veuillez trouver le mode d'emploi en français à:
Podrá encontrar las instrucciones de uso en español en:
Le instruzioni per l'uso sono reperibili in italiano all'indirizzo:
For danske brugsanvisning gå til:
För svensk bruksanvisning gå till:

**http://www.mercodia.se/products/directions-for-use.html**

oder/ou/o/o/eller/eller **Fax No +46 18-570080**

Manufactured by

Mercodia AB, Sylveniusgatan 8A,
SE-754 50 Uppsala,
Sweden

**EXPLANATION OF SYMBOLS USED ON LABELS**

| | |
|---|---|
| $\Sigma$ $\Sigma$ = **96** | Reagents for 96 determinations |
| (hourglass symbol) | Expiry date |
| (thermometer symbol) | Store between 2–8°C |
| **LOT** | Lot No. |
| **IVD** | For *in vitro* diagnostic use |

**INTENDED USE**

Mercodia Ultrasensitive 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 synthesized in the ß-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 Type 1 diabetes mellitus (T1DM) and some other conditions such as hypopituitarism. Insulin levels are raised in Type 2 diabetes Mellitus (T2DM), 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 react with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microplate. 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 of capable of transmitting infections.

## MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader with 450 nm filter
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

## REAGENTS

Each Mercodia Ultrasensitive Insulin ELISA kit 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**<br>Mouse monoclonal anti-insulin | 1 plate | 96 wells<br>8-well strips | Ready for use |
| For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 8 weeks. | | | |
| **Calibrators 1, 2, 3, 4, 5**<br>Recombinant human insulin<br>Color coded yellow<br>Concentration stated on vial label. | 5 vials | 1000 µL | Ready for use |
| **Calibrator 0**<br>Color coded yellow | 1 vial | 5 mL | Ready for use |
| **Enzyme Conjugate 11X**<br>Peroxidase conjugated mouse monoclonal anti-insulin | 1 vial | 1.2 mL | Preparation,<br>see below |
| **Enzyme Conjugate Buffer**<br>Color coded blue | 1 vial | 12 mL | Ready for use |
| **Wash Buffer 21X**<br>Storage after dilution:<br>2-8°C for 8 weeks. | 1 bottle | 50 mL | Dilute with 1000 mL redistilled water to make wash buffer 1X solution. |
| **Substrate TMB**<br>Colorless solution<br>*Note! Light sensitive!* | 1 bottle | 22 mL | Ready for use |
| **Stop Solution**<br>0.5 M $H_2SO_4$ | 1 vial | 7 mL | Ready for use |

## Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer or according to the table below. When preparing enzyme conjugate 1X 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 mL |
| 4 strips | 350 µL | 3.5 mL |

## 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 >20 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 calibrator curve for each assay run.

1. Prepare enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
3. Pipette 25 μL each of Calibrators, controls and samples into appropriate wells.
4. Add 100 μL enzyme conjugate 1X solution to each well.
5. Incubate on a plate shaker (700-900 rpm) for 1 hour at room temperature (18–25°C).
6. Wash 6 times with 700 μL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function, after final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.
   Or manually,
   discard the reaction volume by inverting the microplate over a sink. Add 350 μL wash buffer 1X solution to each well. Discard the wash solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
7. Add 200 μL Substrate TMB into each well.
8. Incubate on the bench for 30 minutes at room temperature (18–25°C)
9. Add 50 μL 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 (Cat no. 10-1134-01/ 10-1164-01) and/or internal serum pools with low, intermediate and high insulin concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, dilution and/or reconstitution dates of kit components, OD values for the blank, Calibrators and controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

## 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 log-log paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.

## Example of results

| Wells | Identity | $A_{450}$ | Mean conc. mU/L |
|---|---|---|---|
| 1A–B | Calibrator 0 | 0.071/0.073 | |
| 1C–D | Calibrator 1* | 0.087/0.088 | |
| 1E–F | Calibrator 2* | 0.186/0.188 | |
| 1G–H | Calibrator 3* | 0.405/0.424 | |
| 2A–B | Calibrator 4* | 1.172/1.241 | |
| 2C–D | Calibrator 5* | 2.262/2.290 | |
| 2E–F | Sample 1 | 0.466/0.470 | 3.5 |
| 2G–H | Sample 2 | 1.987/1.926 | 17.0 |
| 3A-B | Sample 3 | 0.327/0.317 | 2.2 |

*Concentration stated on vial lable.

## Calibrator curve

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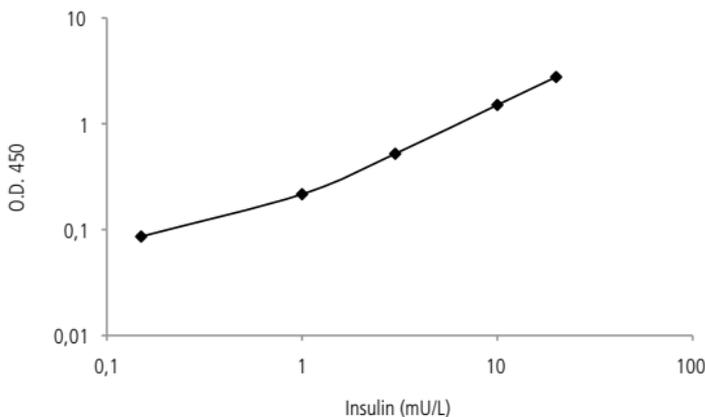


## LIMITATIONS OF THE PROCEDURE

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 hemolyzed samples do not interfere in the assay. However, hemolysis in serum and plasma samples may result in a degradation of insulin which could give falsely low values and contributes to higher inter assay variation. The degradation is dependent on time, temperature and the hemoglobin concentration. Keep hemolyzed samples cold or on ice to prevent the insulin degradation

## 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, were 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

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured. The detection limit is 0,15 mU/L as determined by the methodology described in ISO11843- Part 4.Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

### Recovery

Recovery upon addition is 97-107% (mean 102%).

### Hook effect

Samples with a concentration of up to at least 800 000 mU/L can be measured without giving falsely low results.

### Precision

Each sample was analyzed in 6 replicates on 6 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

| | |
|---|---|
| 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 Lily) | < 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% |
| Ovine insulin | 48% |
| Bovine insulin | 31% |

## CALIBRATION

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

## CONVERSION FACTOR

1 µg/L = 23 mU/L; 1 mU/L = 6.0 pmol/L

## 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.

## REFERENCES

Craig ME, Howard NJ, Silink M, Rawlinson WD. (2003) Reduced frequency of HLA DRB1*03-DQB1*02 in children with type 1 diabetes associated with enterovirus RNA. *J Infect Dis* 187:1562-1570

Gaines-Das, R.E. and Bristow, A.F. (1988) WHO International reference reagents for human proinsulin and human C-peptide. *J Biol Stand* 16: 179-186

Herkner H, Klein N, Joukhadar C, Lackner E, Langenberger H, Frossard M, Bieglmayer C, Wagner O, Roden M and Muller M. (2003) Transcapillary insulin transfer in human skeletal muscle. *Eur J Clin Invest* 33:141-146

Sjostrand M, Gudbjornsdottir S, Holmang A, Lonn L, Strindberg L, Lonnroth P. (2002) Delayed transcapillary transport of insulin to muscle interstitial fluid in obese subjects. *Diabetes* 51:2742-2748

Thule PM, Campbell AG, Kleinhenz DJ, Olson DE, Boutwell JJ, Sutliff RL, Hart CM. (2006) Hepatic Insulin Gene Therapy Prevents Deterioration of Vascular Function and Improves Adipocytokine Profile in STZ-Diabetic Rats. *Am J Physiol Endocrinol Metab* 290:144-122

Zhou J, Pineyro MA, Wang X, Doyle ME, Egan JM. (2002) Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: involvement of PDX-1 and HNF3beta transcription factors. *J Cell Physiol* 192:304-314

Further references can be found on our website: www.mercodia.com

# SUMMARY OF PROTOCOL SHEET

## Mercodia Ultrasensitive Insulin ELISA

| | |
|---|---|
| Add Calibrators, controls* and samples | 25 µL |
| Add enzyme conjugate 1X solution | 100 µL |
| Incubate | 1 hour at 18-25°C on a plate shaker (700-900 rpm) |
| Wash with wash buffer 1X solution | 700 µL, 6 times |
| Add Substrate TMB | 200 µL |
| Incubate | 30 minutes at 18-25°C |
| Add Stop Solution | 50 µL Shake for 5 seconds to ensure mixing |
| Measure $A_{450}$ | Evaluate results |

*not provided        For full details see page 6