Mercodia
Developing diagnostics

Mercodia Iso-Insulin ELISA

Directions for Use

10-1128-01
REAGENTS FOR 96 DETERMINATIONS

CE

IVD

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Manufactured by

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EXPLANATION OF SYMBOLS USED ON LABELS

	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
	Lot No.
	For *in vitro* diagnostic use

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 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 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 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 solution 1X, 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 (Recommended velocity is 700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

REAGENTS

Each Mercodia Iso-Insulin ELISA kit (10-1128-01) contains reagents for 96 wells, sufficient for 43 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 For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 8 weeks.	1 plate	96 wells 8-well strips	Ready for use
Calibrators 1, 2, 3, 4 Recombinant human insulin Color coded yellow Concentration stated on vial label	4 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	600 µL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	6 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 8 weeks	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution.
Substrate TMB Colorless solution *Note! Light sensitive!*	1 bottle	22 mL	Ready for use
Stop Solution 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.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	350 µL	3.5 mL
4 strips	200 µL	2 mL

Storage after dilution: 2-8°C for 8 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

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 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 50 µL enzyme conjugate 1X solution to each well.
5. Incubate on a plate shaker for 1 hour (700-900 rpm) 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 buffer 1X 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.
8. Incubate for 15 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.
    The plate must be 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 (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, preparation 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, verus 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.

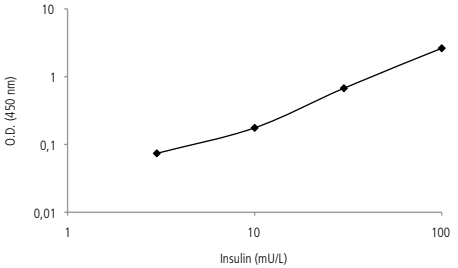
Example of results

Wells	Identity	A_{450}	Mean conc. mU/L
1A–B	Calibrator 0	0.066/0.067	
1C–D	Calibrator 1*	0.084/0.087	
1E–F	Calibrator 2*	0.161/0.165	
1G–H	Calibrator 3*	0.595/0.599	
2A–B	Calibrator 4*	2.377/2.347	
2C–D	Sample 1	0.270/0.272	16.4
2E–F	Sample 2	1.146/1.192	53.6
2G–H	Sample 3	2.044/2.150	92.7

*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.

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 8 different occasions.

		Coefficient of variation		
Sample	Mean value mU/L	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	89%
Insulin aspart	80%
Insulin detemir	22%
Insulin glargin	44%
Insulin glulisine	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%
Ovine insulin	131%
Bovine insulin	58%

CALIBRATION

Mercodia Iso-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 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.

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REFERENCES

Hedman CA, Lindstrom T and Arnqvist HJ (2001) 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

Heise T, Nosek L, Biilmann Ronn B, Endahl L, Heinemann L, Kapitza C and Draeger E (2004) 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

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

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

SUMMARY PROTOCOL SHEET

Mercodia Iso-Insulin ELISA

Add Calibrators, controls* and samples	25 µL
Add enzyme conjugate 1X solution	50 µL
Incubate	1 hour at 18-25°C on a plate shaker, 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add Substrate TMB	200 µL
Incubate	15 minutes at 18-25°C
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A_{450}	450_{nm}

*not provided

For full details see page 6