Mercodia Iso-Insulin ELISA

Directions for Use

10-1128-01
Reagents for 96 determinations

For *in vitro* diagnostic use in EU/EEA, UK, US and Canada

IVD

CE

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https://www.mercodia.com/product/iso-insulin-elisa/
oder/ou/o/eller/of/lub **email: info-global@mercodia.com**

Regulatory status in the rest of the world: For research use only.
Not for use in diagnostic procedures.

Manufactured by

Mercodia AB
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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

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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 synthezised 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 in EU/EEA, UK, US and Canada. Not for internal or external use in humans or animals.
- Regulatory status in the rest of the world: For Research use only. Not for use in diagnostic procedures
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.
- The Stop Solution contains <5% Sulphuric acid.
  The Stop Solution is labeled:

Danger

H318 – Causes serious eye damage.
H315 – Causes skin irritation.
P280 – Wear protective gloves. Wear eye or face protection.
P264 – Wash hands thoroughly after handling.
P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.
P332 + P313 - If skin irritation occurs: Get medical attention.
P305 + P351 + P338 + P310 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

- The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4 and Wash Buffer contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

  The Enzyme Conjugate Buffer, the Calibrators and Wash Buffer are labeled:

Warning

H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

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	1 plate	96 wells 8-well strips	Ready for use
For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 2 months.			
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 2 months	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 2 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 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.

Preparation of samples

No dilution is normally required for serum and plasma samples. However, samples with a concentration above Calibrator 4 should be diluted in Calibrator 0 (or Diabetes Sample Buffer, 10-1195-01).

Test procedure

All reagents and samples must be brought to room temperature before use. Prepare a calibrator curve for each assay run. The product has been optimized and validated without plate sealer.

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 on the bench 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! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution.

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

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.

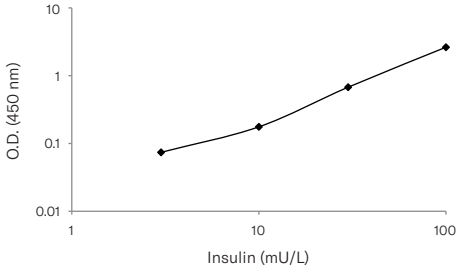
Example of results

Wells	Identity	$A_{450\ nm}$	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 label

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 hemolyzed samples do not interfere in the assay. However, hemo- lysis 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 2 mU/L as determined with the methodology described in ISO11843-Part 4. Detection limit should be seen as part of a method validation, rather than the lowest concentration that can be measured. Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less than or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition was calculated to 92%-97% (mean 94%).
Recovery upon dilution was calculated to 96%-114% (mean 103%).

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 25 different occasions.

		Coefficient of variation	
Sample	Mean value mU/L	Repeatability %*	Within laboratory %**
1	10.3	2.2	4.3
2	37.6	2.9	2.3
3	70.0	1.7	2.2

*Within assay variation
**Total assay variation

Specificity

The following cross reactions have been found:

	Crossreaction
Insulin	100%
Insulin lispro	112%
Insulin aspart	100%
Insulin detemir	28%
Insulin glargine	58%
Insulin glargine M1	47%
Insulin glargine M2	32%
Insulin glulisine	123%
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	76%
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.

Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

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**

Notes

Notes

Notes

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment: **Date:** **KIT LOT#:**

X-O Graf Tryckeri AB

Summary of 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 $A_{450\ nm}$	Evaluate results

*not included

For full details see page 7

For technical support please contact: support@mercodia.com

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