Mercodia

Mercodia Rat Insulin ELISA

Directions for Use

10-1250-01
Reagents for 96 determinations

10-1250-10
Reagents for 10 X 96 determinations

For Research Use Only

Please note that lot-specific Calibrator concentration is stated on vial label

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.

Intended Use

Mercodia Rat Insulin ELISA provides a method for the quantitative determination of insulin in rat 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 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 the B chain. 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.

Principle of the procedure

Mercodia Rat 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 micro-titer 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 research use only.
- Not for internal or external use in humans or animals.
- 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, 5 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 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagents preparations
- Magnetic stirrer
- Vortex mixer
- Redistilled water
- Microplate shaker (Orbital movement at 700-900 cycles per minute)
- Microplate washing device with overflow function (recommended but not required)
- Microplate absorbance reader (450 nm filter)

Reagents for 1 X 96 kit

Each Mercodia Rat Insulin ELISA kit (10-1250-01) 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 For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 2 months.	1 plate	96 wells 8-well strips	Ready for use
Calibrators 1, 2, 3, 4, 5 Rat insulin Color coded yellow Concentration stated on vial label	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	1.3 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	13 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 mixing 100 µL Enzyme Conjugate 11X with 1000 µL Enzyme Conjugate buffer (1+10) for each strip or as in 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
6 strips	600 µL	6 mL
4 strips	400 µL	4 mL

Storage after dilution: 2–8°C for 2 months.

Reagents for 10 X 96 kit

Each Mercodia Rat Insulin ELISA kit (10-1250-10) contains reagents for 10 x 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 For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 2 months.	10 plates	96 wells 8-well strips	Ready for use
Calibrators 1, 2, 3, 4, 5 Rat insulin Color coded yellow Concentration stated on vial label	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 vial	120 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 2 months	2 bottles	200 mL	Preparation, see below
Substrate TMB Colorless solution *Note! Light sensitive!*	1 bottle	220 mL	Ready for use
Stop Solution 0.5 M H_2SO_4	1 vial	70 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 in Enzyme Conjugate buffer 1+10 according to the table below. Mix gently.

Number of plates	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
10 plates	1 vial	1 vial
5 plates	6000 µL	60 mL
3 plates	3600 µL	36 mL
2 plates	2400 µL	24 mL
1 plate	1200 µL	12 mL

Storage after dilution: 2–8°C for 2 months.

Preparation of wash buffer 1X solution

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

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 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. 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 5 should be diluted in Calibrator 0 (or Mercodia 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 (according to the table on previous page).
2. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
3. Pipette 10 µL each of Calibrators, controls and samples into appropriate wells.
4. Add 100 µL of enzyme conjugate 1X solution into each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours 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 15 minutes on the bench at room temperature (18-25°C).
9. Add 50 µL Stop Solution to each well.
   Place the plate on the shaker for approximately 5 seconds to ensure mixing.
10. Read optical density at 450 nm and calculate results.
    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 Rat and Mouse, Low, Medium, and High (10-1220-01) 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 Calibrator 0, Calibrators and Controls.

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.

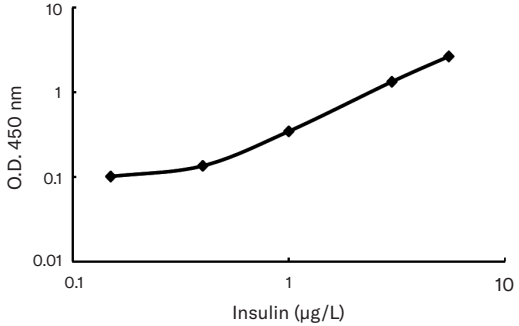
Example of results

Wells	Identity	$A_{450\ nm}$	Mean conc. µg/L
1A–B	Calibrator 0	0.088/0.084	
1C–D	Calibrator 1*	0.097/0.105	
1E–F	Calibrator 2*	0.133/0.136	
1G–H	Calibrator 3*	0.351/0.338	
2A–B	Calibrator 4*	1.350/1.317	
2C–D	Calibrator 5*	2.627/2.668	
2E–F	Sample 1	0.162/0.157	0.49
2G–H	Sample 2	0.324/0.312	0.94
3A–B	Sample 3	1.304/1.202	2.9

*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.



Conversion factor

1 µg corresponds to 174 pmol.

Limitations of the procedure

Performance limitations

Grossly lipemic, icteric or hemolysed samples do not interfere in the assay. Insulin is, however, degraded over time in heamolyzed samples. The degradation could give falsely low values and contributes to higher inter assay variation.

Expected values

Good practice dictates that each laboratory establishes its own expected range of values.

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 µg/L as determined with the methodology described in ISO11843-Part 4.

Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition is 80%-93% (mean 86%).
Recovery upon dilution is 83%-111% (mean 96%).

Hook effect

Samples with a concentration up to at least 450 µg/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 16 different occasions.

		Coefficient of variation		
Sample	Mean value µg/L	within assay %	between assay %	total assay %
1	0.47	5.1	10	11
2	0.96	3.1	4.4	4.7
3	2.8	2.8	3.2	3.5

Specificity

The following cross reactions have been found:

	Crossreaction
Rat C-peptide	< 0.001%
Rat proinsulin	7%
Human insulin	167%
Human proinsulin	75%
Human C-peptide	< 0.05%
Insulin lispro	167%
IGF-I	< 0.02%
IGF-II	< 0.02%
Mouse insulin	75%
Ovine insulin	179%
Bovine insulin	78%

Calibration

Mercodia Rat Insulin ELISA is calibrated against an in-house reference preparation of rat insulin.

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.

References

Korner J, Savontaus E, Chua SC, Jr., Leibel RL and Wardlaw SL (2001) Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. *J Neuroendocrinol* 13:959-966.

Olsson R and Carlsson PO (2005) Better vascular engraftment and function in pancreatic islets transplanted without prior culture. *Diabetologia* 48:469-476.

Rydtren T and Sandler S (2002) Efficacy of 1400 W, a novel inhibitor of inducible nitric oxide synthase, in preventing interleukin-1beta-induced suppression of pancreatic islet function in vitro and multiple low-dose streptozotocin-induced diabetes in vivo. *Eur J Endocrinol* 147:543-551.

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

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#:**

Summary of protocol sheet
Mercodia Rat Insulin ELISA

Add Calibrators, controls* and samples	10 µL
Add enzyme conjugate 1X solution to all wells	100 µL
Incubate	2 hours 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 provided

For full details see page 8

For technical support please contact: support@mercodia.com

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