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Developing diagnostics

Mercodia High Range Rat Insulin ELISA

Directions for Use

10-1145-01
REAGENTS FOR 96 DETERMINATIONS

10-1145-10
REAGENTS FOR 10 X 96 DETERMINATIONS

For Research Use only

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.

INTENDED USE

Mercodia High Range 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 High Range 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 the 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 research use only.
- Not for internal or external use in humans or animals.
- The content 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 samples should be handled as capable of transmitting disease.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 10, 50 and 200 µL (repeat pipettes preferred for addition of enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Microplate reader with 450 nm filter
- Wash device for microtitration plates
- Tube (10-100 mL) for preparation of enzyme conjugate 1X solution
- 1000 mL/10 L bottle
- Redistilled water
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)

REAGENTS FOR 1 X 96 KIT

Each Mercodia High Range Rat Insulin ELISA kit (10-1145-01) 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	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 8 weeks.			
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	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 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
6 strips	300 µL	3.0 mL
4 strips	200 µL	2.0 mL

Storage after dilution: 2-8°C for8 weeks.

REAGENTS FOR 10 X 96 KIT

Each Mercodia High Range Rat Insulin ELISA kit (10-1145-10) 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	10 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 8 weeks.			
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	6 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	60 mL	Ready for use
Wash Buffer 21X	2 bottle	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 bottle	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	3.0 mL	30 mL
3 plates	1.8 mL	18 mL
2 plates	1.2 mL	12 mL
1 plate	600 µL	6 mL

Storage after dilution: 2-8°C for 8 weeks.

Preparation of wash buffer 1X solution

Prepare the needed volume of wash buffer 1X solution by dilution of Wash Buffer 21X in re-distilled water 1+20 according to the table below. Mix properly.

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 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

No dilution is normally required, however, samples containing >150 µg/L should be diluted 1/10 v/v with Calibrator 0.

Note! Buffers containing sodium azide (NaN_3) *can not* be used for sample dilution.

TEST PROCEDURE

Prepare a calibrator curve for each assay run. All reagents and samples must be brought to room tempearture before use.

1. Prepare enzyme conjugate 1X solution (according to the tables on previous pages), wash buffer 1X solution and samples.
2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
3. Pipette 10 µL each of Calibrators and samples into appropriate wells.
4. Add 50 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. Do not include soak step in washing procedure.
   Or manually,
   Discard the reaction volume by inverting the microplate over a sink. Add 350µL 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 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! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

INTERNAL QUALITY CONTROL

Commercial controls 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.

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 for 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.

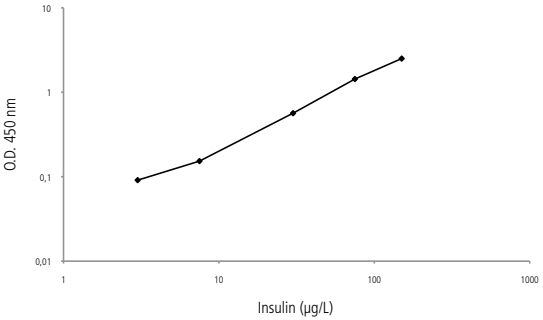
Example of results

Wells	Identity	A_{450}	Mean conc. µg/L
1A–B	Calibrator 0	0.070/0.072	
1C–D	Calibrator 1*	0.115/0.116	
1E–F	Calibrator 2*	0.194/0.200	
1G–H	Calibrator 3*	0.567/0.580	
2A–B	Calibrator 4*	1.310/1.321	
2C–D	Calibrator 5*	2.480/2.452	
2E–F	Sample 1	0.601/0.618	32.2
2G–H	Sample 2	0.330/0.331	15.3

* Concentration stated on vial label.

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 0.174 nmol:

µg/lL	3	7.5	30	75	150
nmol/L	0.52	1.30	5.22	13.05	26.1

LIMITATIONS OF PROCEDURE

Performance limitations

Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

EXPECTED VALUES

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

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit is 1.5 µg/L calculated as two standard deviations above the Calibrator 0.

Recovery

Recovery upon addition is 106 %.
Recovery upon dilution is 100 %.

Hook effect

Samples with a concentration of up to at least 576 µg/L can be measured without giving falsely low results.

Precision [1,2]

Each sample was analyzed in 4 replicates on 5 different occasions. The analysis were done in one laboratory with 1 kit lot and by 1 technician.

Sample	Mean value µg/L	Coefficient of variation within assay %	between assay %	total assay %
1	16.2	4.5	4.1	6.1
2	32.7	4.8	2.5	5.4

[1] ISO 5725-1, [2] EP-5A

Reproducebillity [1,2]

Each sample was analyzed in 4 replicates on 107 different occasions. The analysis were done in two laboratories with 18 kit lots and by 6 technicians.

Sample	Mean value µg/L	Coefficient of variation within assay %	between assay %	total assay %
1	14.3	4.6	3.4	3.9
2	30.8	3.9	2.9	3.7

[1] ISO 5725-1, [2] EP-5A

SPECIFICITY

IGF-I	<0.02%
IGF-II	<0.02%
Mouse C-peptide I	< 0.002%
Mouse C-peptide II	< 0.001%
Rat C-peptide I	<0.03%
Rat C-peptide II	<0.03%
Mouse insulin	75%
Rat insulin	100%
Mouse Proinsulin I	33%
Mouse Proinsulin II	51%
Rat Proinsulin I	8%
Rat Proinsulin II	51%
Bovine insulin	78%
Porcine insulin	476%
Ovine insulin	179%
Human C-peptide	<0.05%
Human insulin	167%
Human proinsulin	75%
Insulin lispro (Humalog® Eli Lilly)	167%

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

Kullin M *et al.* (2003) Protection of rat pancreatic islets by potassium channel openers against alloxan, sodium nitroprusside and interleukin-1beta mediated suppression-possible involvement of the mitochondrial membrane potential. *Diabetologia* 46:80-88

von Mach MA *et al.* (2003) Cryopreservation of islets of Langerhans: Optimization of protocols using rat pancreatic tissue. *EXCLI Journal* 2:6-21

SUMMARY PROTOCOL SHEET

Mercodia High Range Rat Insulin ELISA

Add Calibrators and samples	10 µl
Add enzyme conjugate 1X solution	50 µl
Incubate	2 hours at 18-25°C on a plate shaker
Wash	6 times
Add Substrate TMB	200 µl
Incubate	15 minutes
Add Stop Solution	50 µl Shake for 5 seconds to ensure mixing
Measure A_{450}	Evaluate results

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Version 5.0