Mercodia
Immunoassays & Services

Mercodia Equine Insulin ELISA

Directions for Use

10-1205-01
REAGENTS FOR 96 DETERMINATIONS

For Research Use Only

Manufactured by

Mercodia AB
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SE-754 50 Uppsala
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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 Equine Insulin ELISA provides a method for the quantitative determination of insulin in equine serum and 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.

Matching energy intake with the energy expenditure is important for the exercising horse (1-2). Parameters such as the hormones ghrelin, adiponectin and insulin have shown to play a major role in mediating the energy balance either through their effects on feed intake or metabolic regulation. Glucose and insulin responses are modified by the diet and obesity, exercise level and stress also alters glucose and insulin metabolism (1-3).

PRINCIPLE OF THE PROCEDURE

Mercodia Equine 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 microplate. After a simple washing step that removes unbound enzyme labelled antibody, the bound conjugate is detected by reaction with 3,3´-5,5´-tetramethylbenzidine (TMB). The reaction is stopped by the addition of acid, giving a colorimetric endpoint that can be read spectrophotometrically.

WARNINGS AND PRECAUTIONS

- The contents of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- All samples should be handled as capable of transmitting infections.
- Not for internal or external use in humans or animals.
- Each well can only be used once.
- For Research Use Only.
- 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 wa ter. 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 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 Equine Insulin ELISA (10-1205-01) 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 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, 5 Porcine 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 dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer according to the table below. Mix gently.
When preparing enzyme conjugate 1X solution for the whole plate or if the reagents are to be used within 2 weeks, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µL	7 mL
4 strips	400 µL	4 mL

Storage after dilution: 2-8°C for 2 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, citrate 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 or plasma. All samples containing equine insulin above the highest calibrator should be diluted with Calibrator 0 or with Mercodia Diabetes Sample Buffer (10-1195-01).

TEST PROCEDURE

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

1. Prepare enzyme conjugate 1X solution (according to the table on previous page) 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 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 buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing.
7. Add 200 µL Substrate TMB into each well.
8. Incubate on the bench for 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! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution.

INTERNAL QUALITY CONTROL

Commercial control such as Mercodia Insulin Control Animal Low, Medium and High (10-1221-01) and/or internal serum pools with low, intermediate and high equine 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

The concentration of equine insulin is obtained by computerized data reduction of the absorbance for the Calibrators, except Calibrator 0, versus the concentration using cubic spline regression.

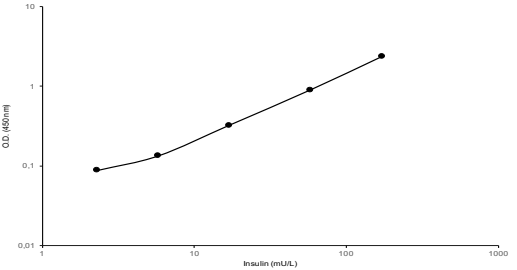
Example of results

Wells	Identity	A_{450}	Mean conc. mU/L
1A-B	Calibrator 0	0.054/0.053	
1C-D	Calibrator 1*	0.090/0.086	
1E-F	Calibrator 2*	0.135/0.133	
1G-H	Calibrator 3*	0.319/0.329	
2A-B	Calibrator 4*	0.907/0.880	
2C-D	Calibrator 5*	2.405/2.304	
2E-F	Sample 1	0.184/0.185	9.1
2G-H	Sample 2	0.571/0.560	33
3A-B	Sample 3	1.597/1.543	112

*Exact concentration indicated 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

Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay. Insulin is, however, degraded over time in hemolyzed samples. The degradation 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.

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 1.15 (mU/L) determined with 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 102-125 % (mean 115 %).
Recovery upon dilution is 78-93 % (mean 86 %).

Hook effect

Samples with a concentration up to 115 000 mU/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 28 different occasions.

		Coefficient of variation	
Sample	Mean value mU/L	Repeatability %*	Within laboratory %**
1	8.6	3.4	4.4
2	34.5	3.0	4.5
3	106	3.2	2.5

*Within assay variation
**Total assay variation

Specificity

The following cross reaction have been fond:

Porcine Insulin	100 %
Porcine C-peptide	< 0.001 %
Porcine Proinsulin	< 0.2 %
Human Insulin	108 %
Human C-peptide	< 0.01 %
Human Proinsulin	< 0.1 %
NovoRapid	3.2 %
Levemir	n.d.
Lantus	27 %
Humalog	n.d.

n.d.=not detected

CALIBRATION

Mercodia Equine Insulin ELISA is calibrated against an inhouse reference preparation of porcine 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 authorized distributors, in such event, shall not be liable for damages indirect of consequential.

REFERENCES

1. Jansson A, Nyman S, Lindholm A, Lindberg JE (2002) Effects on exercise metabolism of varying starch and sugar proportions. *Equine Vet J Suppl* 34:17-21

2. Gordon ME, McKeever KH, Betros CL, Helio C, Filho M (2007) Exercise-induced alterations in plasma concentrations of ghrelin, adiponectin, glucose, insulin, and cortisol in horses. *Vet J* 173(3):532-40

3. Ralston SL (2002) Insulin and glucose regulation. *Vet Clin North Am Equine Pract* 18:295-304

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

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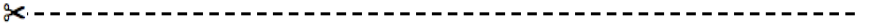

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment: Date: KIT LOT#:

SUMMARY OF PROTOCOL SHEET
Mercodia Equine Insulin ELISA

Add Calibrators, controls* and samples	25 µL
Add enzyme conjugate 1X solution	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}	Evaluate results

*not included

For full details see page 7

For technical support please contact support@mercodia.com.

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