Mercodia
Developing diagnostics

Mercodia Feline Insulin ELISA

Directions for Use

10-1233-01
REAGENTS FOR 96 DETERMINATIONS

Manufactured by

Mercodia AB,
Sylveniusgatan 8A,
SE-754 50 Uppsala,
Sweden

EXPLANATION OF SYMBOLS USED ON LABELS

	Reagents for 96 determinations
	Expiry date
	Store between 2-8°C
	Lot No.

INTENDED USE

Mercodia Feline Insulin ELISA provides a method for the direct quantitative determination of insulin levels in feline serum or plasma samples.

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 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.

Diabetes mellitus is one of the most common endocrine disorder in cats, with a form that closely resembles human type 2 diabetes (1-2). Its incidence rate among cats appears to be increasing, probably due to an increase in obesity and a decrease in physical activation in the cat population (1-2). Obesity increases the risk for diabetes 3- to 5-fold (2). Diabetes occurs in a wide range of cats, but most cats are over six years of age when diagnosed (1-2). Diabetic cats may go into remission and studies have shown that different insulin therapy treatments may have an influence on this (3).

PRINCIPLE OF THE PROCEDURE

Mercodia Feline 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. 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 content of this kit and their residues must not be allowed to come into contact with ruminating animal or swine.
- The Stop Solution in this kit contains 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All patient samples should be handled as capable of transmitting infections.

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 Feline Insulin ELISA 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 and store at 2-8°C for 8 weeks.			
Calibrators 1, 2, 3, 4, 5 Feline insulin Color coded yellow Concentration stated on vial label.	5 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial.
Storage after reconstitution: 2-8°C for 8 weeks. For storage of reconstituted Calibrators for more than 8 weeks, store at –20°C.			
Calibrator 0 Color coded yellow	1 vial	5 mL	Ready for use
Enzyme Conjugate 11X Peroxidase conjugated mouse monoclonal anti-insulin	1 vial	2.5 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	25 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. Mix gently.
When preparing enzyme conjugate 1X solution for the whole plate or if the reagents are to be used within two weeks, pour all of the Enzyme Conjugate 11X into the Enzyme Conjugate Buffer vial.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
6 strips	1000 µL	10 mL
4 strips	800 µL	8 mL

Storage after dilution: 2-8°C for two 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. 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 >700 ng/L should be diluted at least 1/10 v/v with Calibrator 0.
Note! Buffers containing sodium azide (NaN_3) can not be used for sample dilution.

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.

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 and samples in duplicate.
3. Pipette 10 µL each of Calibrators and samples into appropriate wells.
4. Add 200 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 procedure.
7. Add 200 µL Subtrate TMB into each well.
8. Incubate for 30 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 feline 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 feline insulin is obtained by computerized data reduction of the absorbance for the Calibrators, except Calibrator 0, versus the concentration using cubic spline regression.

Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the feline Insulin concentration on a lin-lin paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.

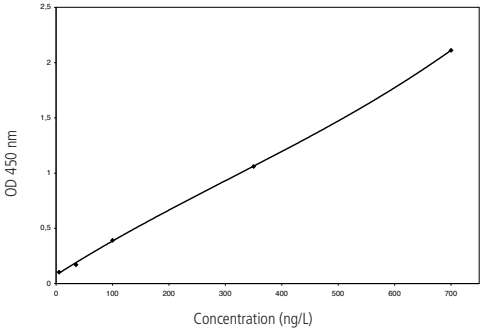
Example of results

Wells	Identity	A_{450}	Mean conc. ng/L
1A-B	Calibrator 0	0.100/0.084	
1C-D	Calibrator 1*	0.120/0.112	
1E-F	Calibrator 2*	0.193/0.184	
1G-H	Calibrator 3*	0.368/0.356	
2A-B	Calibrator 4*	1.076/0.969	
2C-D	Calibrator 5*	2.293/2.157	
2E-F	Sample 1	0.177/0.170	28.9
2G-H	Sample 2	0.334/0.328	94.3
3A-B	Sample 3	0.768/0.761	254

*Exact 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

Grossly lipemic, icteric or haemolyzed samples do not interfere in the assay.
However, hemolysis in serum and plasma samples may result in a degradation of insulin. 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 ≤ 9.2 (ng/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 as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition is 84-101% (mean 95%).
Recovery upon dilution is 91-128% (mean 107%).

Hook effect

Samples with a concentration of up to 27 600 µg/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 16 different occasions.
The analysis were done in 2 laboratories with 2 kit lots and by 4 technicians.

		Coefficient of variation		
Sample	Mean value (ng/l)	within assay %	between assay %	total assay %
1	31.2	6.8	12.5	13.0
2	88.6	4.3	7.3	7.7
3	257	6.1	6.7	7.4

Specificity
The following cross-reactions have been found:
NovoRapid® (Insulin aspart) 7.4%
Levemir® (Insulin detemir) < 0.09%
Lantus® (Insulin glargin) 10.6%
Humalog® (Insulin lispro) < 0.00000003%
Apidra® (Insulin glulisine) < 0.0000009%
Vetsulin®, Caninsulin® 72%

CALIBRATION
Mercodia Feline Insulin ELISA is calibrated against an in house reference preparation of feline 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. Rand JS, Fleeman LM, Farrow HE, Appleton DJ, Lederer R (2004) Canine and Feline Diabetes Mellitus: Nature or Nurture? *J Nutr* 134:2072S-2080S

2. Hoenig M (2002) Comparative aspects of diabetes mellitus in dogs and cats. *Mol Cell Endocrin* 197:221-229

3. Michiels L, Reusch CE, Boari A, Petrie G, Mandigers P, Thollot IG, Rosenberg D, Mooney C, Bonfanti U, Font A, Sparkes A, Bewig K, Clercx C, Jensen AL, Horspool LJI (2008) Treatment with 46 cats with porcine lente insulin – a prospective, multicentre study. *J Fel Med Sur* 10:439-451

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

SUMMARY OF PROTOCOL SHEET

Add Calibrators, controls and samples	10 µL
Add enzyme conjugate 1X solution	200 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	30 minutes at 18-25°C
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A_{450}	Evaluate results

*not provided

For full details see under Test Procedure