Mercodia
Developing diagnostics

# Mercodia Ovine Insulin ELISA

Directions for Use

**10-1202-01**
**REAGENTS FOR 96 DETERMINATIONS**

Manufactured by

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## SUMMARY OF PROTOCOL SHEET

| | |
|---|---|
| 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 | 700µL, 6 times |
| Add Substrate TMB | 200 µL |
| Incubate | 15 minutes at 18-25°C, 700-900 rpm |
| Add Stop Solution | 50 µL<br>Shake for 5 seconds to ensure mixing |
| Measure $A_{450}$ | Evaluate results |

*not provided — For full details see under Test Procedure

## EXPLANATION OF SYMBOLS USED ON LABELS

| | |
|---|---|
| $\Sigma$ = 96 | Reagents for 96 determinations |
| | Expiry date |
| | Store between 2-8°C |
| LOT | Lot No. |



31-3161
Version 3.0

## INTENDED USE

Mercodia Ovine Insulin ELISA provides a method for the quantitative determination of insulin in ovine 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.

Extensive research on how to improve the nutritional, metabolic and health status of ruminants has been at focus for a long time. A decrease in dry matter intake is a major physiological change in ruminants. This may lead to several metabolic disorders such as ketosis, fatty liver and hypocalcemia (1-2). Food intake is a complex mechanism, regulated by several factors including for example hormones, metabolites and environmental factors (1-3).

## PRINCIPLE OF THE PROCEDURE

Mercodia Ovine 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.
- Not for internal or external use in humans or animals.

## 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 washing device with overflow function (recommended but not required)

## REAGENTS

Each Mercodia Ovine 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**<br>Mouse monoclonal anti-insulin<br>For unused microtitration strips, reseal the bag using adhesive tape and store at 2-8°C within 8 weeks | 1 plate | 96 wells<br>8-well strips | Ready for use |
| **Calibrators 1, 2, 3, 4, 5**<br>Ovine insulin<br>Concentration stated on vial label.<br>Color coded yellow | 5 vials | 1000 µL | Ready for use |
| **Calibrator 0**<br>Color coded yellow | 1 vial | 5 mL | Ready for use |
| **Enzyme Conjugate 11X**<br>Peroxidase conjugated mouse monoclonal anti-insulin | 1 vial | 1.3 mL | Preparation, see below |
| **Enzyme Conjugate Buffer**<br>Color coded blue | 1 vial | 13 mL | Ready for use |
| **Wash Buffer 21X**<br>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**<br>Colorless solution<br>*Note! Light sensitive!* | 1 bottle | 22 mL | Ready for use |
| **Stop Solution**<br>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 |
| 6 strips | 500 µL | 5 mL |
| 4 strips | 400 µL | 4 mL |

Storage after dilution: 2-8°C. Use within 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. 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 ovine insulin above the highest calibrator should be diluted with Calibrator 0 or with Mercodia Diabetes Sample Buffer 10-1195-01.

## INTERNAL QUALITY CONTROL

Commercial control and/or internal serum pools with low, intermediate and high ovine insulin concentrations should routinely be assayed as unknowns, 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.

## 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, 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 procedure.
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!* To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

## CALCULATION OF RESULTS

### Computerized calculation

The concentration of ovine 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 ovine insulin concentration on a lin-lin paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.

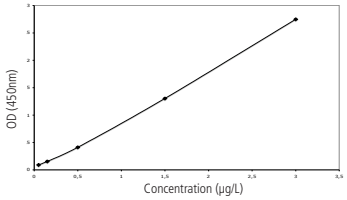
**Example of results**

| Wells | Identity | $A_{450}$ | Mean conc. µg/L |
|---|---|---|---|
| 1A-B | Calibrator 0 | 0.058/0.059 | |
| 1C-D | Calibrator 1* | 0.093/0.090 | |
| 1E-F | Calibrator 2* | 0.157/0.155 | |
| 1G-H | Calibrator 3* | 0.412/0.414 | |
| 2A-B | Calibrator 4* | 1.301/1.309 | |
| 2C-D | Calibrator 5* | 2.765/2.735 | |
| 2E-F | Sample 1 | 0.193/0.190 | 0.206 |
| 2G-H | Sample 2 | 0.356/0.361 | 0.437 |
| 3A-B | Sample 3 | 0.788/0.764 | 0.921 |

*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 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.

## 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 0.025 (µg/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 94-114 % (mean 103 %).
Recovery upon dilution is 68-122 % (mean 89 %).

### Hook effect

Samples with a concentration of up to 1 000 µg/L can be measured without giving falsely low results.

### Precision

Each sample was analyzed in 4 replicates on 19 different occasions.

| Sample | Mean value (µg/L) | Coefficient of variation | | |
|---|---|---|---|---|
| | | within assay % | between assay % | total assay % |
| 1 | 0.201 | 3.7 | 6.5 | 6.8 |
| 2 | 0.403 | 1.2 | 4.5 | 4.6 |
| 3 | 0.859 | 1.7 | 4.7 | 4.8 |

### Specificity

The following cross reaction have been fond:

| | | | |
|---|---|---|---|
| NovoRapid | 2.0 % | Lantus | 21 % |
| Humalog | < 0.000001 % | Levemir | < 0.0000004 % |

## CALIBRATION

Mercodia Ovine Insulin ELISA is calibrated against an in-house reference preparation of ovine 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

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