DRG® Ovine Insulin ELISA (EIA-4739)

As of July 2007 **For Veterinary Use Only**

INTENDED USE

The Insulin (Ovine) 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 β-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

The 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.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100, 200 and 1000 µl (repeat pipettes preferred for addition of enzyme conjugate solution, TMB Substrate and Stop Solution)

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- Beakers and cylinders for reagent preparation
- Redistilled water
- Microplate reader (450 nm filter)
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device

REAGENTS

Each Ovine Insulin ELISA kit contains reagents for 96 wells, sufficient for 42 samples and one standard 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, 8-well strips	96 wells	Ready for use
For unused microtitration strips, reseal the bag using adhesive tape and store at 2-8°C for 2 months.			
Standards 1, 2, 3, 4, 5 (Ovine insulin) Concentration stated on vial label.	5 vials	1000 µl	Ready for Use
Standard 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	40 ml	Dilute with 800 ml redistilled water to make wash buffer.
TMB Substrate Solution (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

1.1 Preparation of Enzyme Conjugate Colution

Prepare the needed volume of enzyme conjugate solution by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer according to the table below. Mix gently.

When preparing enzyme conjugate 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 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. 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.

1.2 Preparation of Samples

No dilution is normally required for serum or plasma.

All samples containing Ovine insulin above the highest standard should be diluted with Standard 0 or with Diabetes Sample Buffer (REF: 10-1195-01).

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Perform each determination in duplicate for standards and unknowns. Prepare a standard curve for each assay run.

1. Prepare enzyme conjugate solution (according to the table on previous page) and wash buffer.
2. Prepare sufficient microplate wells to accommodate Standards and samples in duplicate.
3. Pipette 25 µl each of Standards and samples into appropriate wells.
4. Add 100 µl of enzyme conjugate solution into each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours at room temperature (18-25°C).
6. Wash plate 6 times with 700 µl wash buffer per well with an automatic washer. *
   After final wash, invert and tap the plate against absorbent paper.
7. Add 200 µl TMB Substrate Solution into each well.
8. Incubate 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.

* The plate can be washed with an automatic washer or manually. When washing with an automatic washer please use the overflow function. If there is no overflow function available on the automatic washer please wash manually.

Manual wash can be done either with a pipette or a squirt bottle:
Aspirate the reaction volume and add 400 µl wash buffer to each well with a pipette or fill the wells completely by spraying wash buffer into the wells with a squirt bottle. Aspirate completely and repeat 5 times. The overflow is not a problem rather an advantage.

Note!
To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

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; reconstitution dates of kit components; OD values for the blank, Standards and controls.

CALCULATION OF RESULTS

Computerized calculation

The concentration of ovine insulin is obtained by computerized data reduction of the absorbance for the Standards, except Standard 0, versus the concentration using cubic spline regression.

Manual calculation

1. Plot the absorbance values obtained for the Standards, except Standard 0, against the ovine insulin concentration on a lin-lin paper and construct a standard curve.
2. Read the concentration of the unknown samples from the standard curve.

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Example of results

Wells	Identity	A450	Mean conc. µg/l
1A-B	Standard 0	0.058/0.059	
1C-D	Standard 1 (0.05 µg/l)*	0.093/0.090	
1E-F	Standard 2 (0.15 µg/l)*	0.157/0.155	
1G-H	Standard 3 (0.5 µg/l)*	0.412/0.414	
2A-B	Standard 4 (1.5 µg/l)*	1.301/1.309	
2C-D	Standard 5 (3.0 µg/l *	2.765/2.735	
2E-F	Unknown 1	0.193/0.190	0.206
2G-H	Unknown 2	0.356/0.361	0.437
3A-B	Unknown 3	0.788/0.764	0.921

*Exact concentration indicated on vial label.

LIMITATIONS OF THE PROCEDURE

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

1.3 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 Standard 1 should not be calculated, instead expressed as less or equal to ≤ the concentration indicated on the vial for Standard 1.

1.4 Recovery

Recovery upon addition is 94-114 % (mean 103 %).

Recovery upon dilution is 68-122 % (mean 89 %).

1.5 Hook effect

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

1.6 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

1.7 Specificity

The following cross reactions have been fond:

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

CALIBRATION

The 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 DRG may affect the results, in which event DRG disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. DRG and its authorized distributors, in such event, shall not be liable for damages indirect of consequential.

REFERENCES

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2. McCann JP, Loo SC, Aalseth DL, Abribat T (1997) Differential effects of GH stimulation on fasting and prandial metabolism and plasma IGFs and IGF-binding proteins in lean and obese Ovine. J Endocrin154:329-346
3. Melandez p, Krueger T, White J, Badinga L, Verstegen J, donovan GA, Archbald LF (2006) Effect of ghrelin in dry matter intake and energy metabolism in prepartum Ovine: a preliminary study. Theriogenology 66: 1961-1968

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SUMMARY OF PROTOCOL SHEET

Add Standards and samples	25 µl
Add Enzyme Conjugate solution	100 µl
Incubate	2 hours at 18-25°C on a plate shaker
Wash plate with wash buffer	6 times
Add TMB Substrate Solution	200 µl
Incubate	15 minutes at 18-25°C
Add Stop Solution	50 µl Shake for 5 seconds to ensure mixing
Measure A450	Evaluate results

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Ογκος/αριθ..

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