DRG® Mouse Insulin ELISA (EIA-3439)

REVISED 1 APR. 2010 RM (VERS. 5.0) **FOR VETERINARY USE**

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Mouse Insulin ELISA provides a method for the quantitative determination of insulin in mouse serum or plasma.

2 PRINCIPLE OF THE PROCEDURE

The Mouse 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 microplate wells. 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.

3 WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for Use in Diagnostic Procedures.
- 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 0.5M H_2SO_4. Follow routine precautions for handling hazardous chemicals.

4 MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 200 µl and 1000 µl (Repeating pipettes preferred for addition of enzyme conjugate solution, Substrate TMB and Stop Solution)
- EIA plate reader with 450 nm filter
- Wash device for microtitration plates
- Tube (10–100 ml) for preparation of enzyme conjugate solution
- 1000 ml/10 l bottle
- Redistilled water
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)

5 REAGENTS FOR 1 X 96 KIT

Each Mouse 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, store at 2–8°C and use within 8 weeks.			
Calibrators 1, 2, 3, 4, 5 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 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

5.1 Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by mixing 100 µl Enzyme Conjugate 11X with 1000 µl Enzyme Conjugate buffer (1+10) for each strip or as in 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	600 µl	6.0 ml
4 strips	400 µl	4.0 ml

Storage after dilution: 2–8°C for two months.

6 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.

6.1 Preparation of samples

No dilution is normally required, however, samples containing > 6.5 µg/l should be diluted 1/10 v/v with Calibrator 0 µg/l.

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

7 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 table on previous page), 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 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. Do not include soak step in washing procedure.
   Or manually,
   Discard the reaction volume by inverting the microplate over a sink. Add 350µl wash 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

8 INTERNAL QUALITY CONTROL

Commercial controls such as Diabetes-Antigen Control Rat, Mouse (L, M, H) (CTL-4783) 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.

9 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	0,072/0,073	
1C–D	Calibrator 1*	0,099/0,101	
1E–F	Calibrator 2*	0,136/0,133	
1G–H	Calibrator 3*	0,466/0,479	
2A–B	Calibrator 4*	1,136/1,118	
2C–D	Calibrator 5*	2,901/2,985	
2E–F	Sample 1	0,163/0,170	0,63
2G–H	Sample 2	0,352/0,361	1,2
3A–B	Sample 3	1,468/1,464	3,7

* Concentration stated on vial label.

Conversion factor

1 µg corresponds to 174 pmol.

10 LIMITATIONS OF THE PROCEDURE

Performance limitations

Grossly lipemic, icteric or haemolysed samples do not interfere in the assay. Insulin is, however, degraded over time in haemolyzed samples. The degradation could give falsely low values and contributes to higher inter-assay variation

11 EXPECTED VALUES

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

12 PERFORMANCE CHARACTERISTICS

12.1 Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as a part of a method validation, rather than the lowest concentration that can be measured.

The detection limit is ≤ 0.2 µg/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 less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

12.2 Recovery

Recovery upon addition is 100-130 (113) %.

Recovery upon dilution is 109-149 (129) %.

12.3 Hook effect

Samples with a concentration up to at least 450 µg/l can be measured without giving falsely low results.

12.4 Precision

Each sample was analyzed in 4 replicates on 16 different occasions.

Sample	Mean value µg/l	Coefficient of variation		
		Within assay %	Between assay %	Total assay %
1	0.65	3.1	5.9	6.1
2	1.3	1.9	3.4	3.5
3	3.6	2.9	5.1	5.3

12.5 Specificity

Human insulin	195%
Human proinsulin	82%
Human C-peptide	< 0.05%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	146%
Rat proinsulin	14%
Rat C-peptide	< 0.001%
Porcine insulin	628%
Sheep insulin	256%
Bovine insulin	110%

13 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 authorised distributors, in such event, shall not be liable for damages indirect or consequential.

14 REFERENCES

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15 SUMMARY PROTOCOL SHEET

Add Calibrators, Controls and Samples	10 µl
Add enzyme conjugate 1X solution to all wells	100 µl
Incubate	2 hours at 18–25°C on a shaker
Wash plate with wash buffer 1X solution	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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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	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	Usage 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	Température 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	Vertreiber	Distributeur	Distribuidor	Distributore
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 tilsttrækkeligt 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	Όγκος/αριθ..