DRG® Mouse Insulin ultrasensitive ELISA (EIA-3440)

Revised 17 June 2010 rm (Vers. 4.0) **For Veterinary Use Only**

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

INTENDED USE

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

PRINCIPLE OF THE PROCEDURE

The Ultrasensitive 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 the microtitration well. 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.

WARNINGS AND PRECAUTIONS

- 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 animal or swine.
- The Stop Solution in this kit contains 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All samples should be handled as capable of transmitting infections.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100 and 200 µL (Repeating pipettes preferred for addition of enzyme conjugate solution, Substrate TMB and Stop Solution.)
- 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)

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REAGENTS FOR 1 X 96 KIT

Each Ultrasensitive 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	1 plate	96 wells	Ready for use
(mouse monoclonal anti-insulin)		8-wells strips	

For unused microplate wells, completely reseal the bag using adhesive tape store at 2–8°C and use within 8 weeks

Calibrators 1, 2, 3, 4, 5	5 vials	1000 µl	Ready for use

Color coded yellow

Calibrator 0	1 vial	5 ml	Ready for use

Colour coded yellow

Enzyme Conjugate 11X	1 vial	1.3 mL	Preparation, see below

(Peroxidase conjugated mouse monoclonal anti-insulin)

Enzyme Conjugate Buffer	1 vial	13 ml	Ready for use

Colour coded blue

Wash Buffer 21X	1 bottle	50 ml	Dilute 1+20
Storage after dilution: 2-8°C for 8 weeks.			Dilute with 1000 ml redistilled water to make Wash Buffer 1X solution

Substrate TMB	1 bottle	22 ml	Ready for use

Colorless solution
Note! Light sensitive!

Stop Solution	1 vial	7 ml	Ready for use

0.5 M H_2SO_4

Preparation of enzyme conjugate 1X solution

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. 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.
Enzyme Enzyme Number of strips Conjugate 11X Conjugate Buffer

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 2 months.

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.

Preparation of samples

No dilution is normally required, however, samples containing insulin concentration above the highest Calibrator should be diluted 1/10 v/v with Calibrator 0.
Note! Buffers containing sodium azide (NaN_3) can not be used for sample dilution.

TEST PROCEDURE

Prepare a calibrator curve for each assay run .All reagents and samples must be brought to room temperature 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 25 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 350 µL 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.

INTERNAL QUALITY CONTROL

Commercial controls such as Mercodia Diabetes Antigen Control Rat/Mouse (L, M, H) (Ref.: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.

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 lin-lin paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.

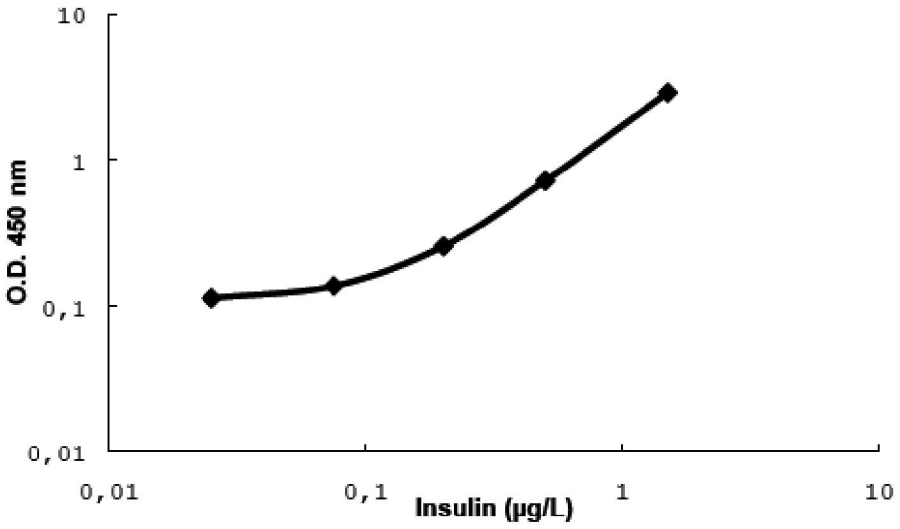
Example of results

Wells	Identity	A450	Mean conc. µg/L
1A–B	Calibrator 0	0.099/0.094	
1C–D	Calibrator 1*	0.115/0.109	
1E–F	Calibrator 2*	0.136/0.136	
1G–H	Calibrator 3*	0.248/0.261	
2A–B	Calibrator 4*	0.703/0.730	
2C–D	Calibrator 5*	2.877/2.881	
2E–F	Sample 1	0.184/0180	0.131
2G–H	Sample 2	0.248/0.260	0.200
3A–B	Sample 3	0.386/0.409	0.308

* Concentration stated on vial label.

Calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



Conversion factor

1 µg corresponds to 174 pmol.

LIMITATIONS OF THE PROCEDURE

Grossly lipemic, icteric or haemolysed samples do not interfere in the assay. Insulin is, however, degraded over time in haemolysed 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 a part of a method validation, rather than the lowest concentration that can be measured.
The detection limit is 0.025 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.

Recovery

Recovery upon addition is 76-94 (mean 85) %.
Recovery upon dilution is 110-132 (mean 123) %.

Hook effect

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

Precision

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

		Coefficient of variation		
Sample	**Mean value µg/L**	**within assay %**	**between assay %**	**total assay %**
1	0,14	2,9	4,9	5,1
2	0,22	1,5	3,9	3,9
3	0,26	2,2	4,5	4,6

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%

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.

REFERENCES / Literature

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   J Clin Invest 111:1555-1562
3. Friesen NT, Buchau AS, Schott-Ohly P, Lgssiar A, Gleichmann H (2004) Generation of hydrogen peroxide and failure of antioxidative responses in pancreatic islets of male C57BL/6 mice are associated with diabetes induced by multiple low doses of streptozotocin.
   Diabetologia 47:676¬685
4. Jaeckel E, Lipes MA, von Boehmer H (2004) Recessive tolerance to preproinsulin 2 reduces but does not abolish type 1 diabetes.
   Nat Immunol 5:1028-1035

SUMMARY PROTOCOL SHEET

Ultrasensitive Mouse Insulin ELISA

Add Calibrators, Controls and samples	**25 µL**
Add enzyme conjugate 1X solution to all wells	100 µL
Incubate	2 hours at 18-25°C on a plate 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