DRG® Ultrasensitive Rat Insulin ELISA (EIA-2943)

REVISED 3 OCT. 2012 RM (VERS. 7.1) **FOR VETERINARY USE ONLY**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

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

1 INTENDED USE

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

2 PRINCIPLE OF THE PROCEDURE

The Ultrasensitive Rat 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 labelled 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 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All samples should be handled as of capable of transmitting infections.

4 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.)
- Microplate reader with 450 nm filter
- Wash device for microplates
- Tube (10 mL) for preparation of enzyme conjugate 1X solution
- 1000 mL bottle
- Redistilled water
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)

5 REAGENTS

Each Ultrasensitive Rat 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-wells 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 indicated on vial label.	5 vials	1000 µl	Ready for use
Calibrator 0 Colour 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 Colour 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 with1000 mL redistilled water to make wash buffer 1X solution
Substrate TMB Colorless solution *Note! Light sensitive!*	1 vial	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 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.

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.

Preparation of samples
No dilution is normally required, however, samples containing >1.0 µg/L should be diluted 1/10 v/v with Calibrator 0.
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 temperature before use.

1. Prepare enzyme conjugate 1X solution (according to the table on previous pages), 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. 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 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) (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 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.

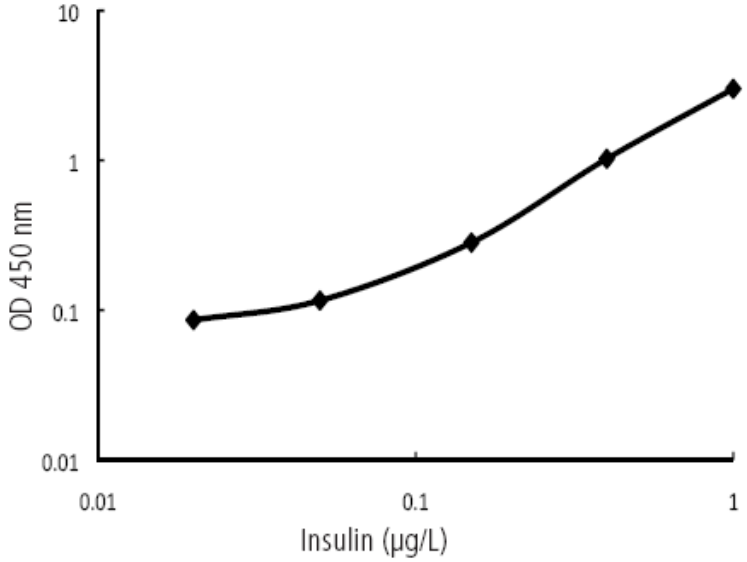
Example of results

Wells	Identity	A_{450}	Mean conc. µg/L
1A–B	Calibrator 0	0.077/0.070	
1C–D	Calibrator 1*	0.087/0.083	
1E–F	Calibrator 2*	0.119/0.115	
1G–H	Calibrator 3*	0.286/0.276	
2A–B	Calibrator 4*	1.063/1.027	
2C–D	Calibrator 5*	2.989/3.033	
2E–F	Unknown 1	0.216/0.210	0.12
2G–H	Unknown 2	0.338/0.339	0.18
3A–B	Unknown 3	0.376/0.369	0.19

*Concentration indicated on vial label

Calibrator curve

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



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 haemolysed samples. The degradation could give falsely low values and contribute to higher inter assay variation.

11 EXPECTED VALUES

Good practice dictates 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 ≤0.020 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 Hook effect

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

12.3 Precision

Each sample was analyzed in four replicates on 22 different occasions.

Sample	Mean value µg/L	Coefficient of variation		
		Within assay %	between assay %	Total assay %
1	0.11	2.9	4.8	5.0
2	0.17	2.0	4.2	4.3
3	0.19	2.6	3.5	3.8

12.4 Specificity

Human insulin	**167%**
Human proinsulin	75%
Human C-peptide	< 0,05 %
Insulin lispro (Humalog®)	167%
IGF-I	< 0,02 %
IGF-II	< 0,02 %
Rat proinsulin I	8%
Rat proinsulin II	51%
Mouse proinsulin I	33%
Mouse proinsulin II	51%
Mouse c-peptide I	<0,002 %
Mouse c-peptide II	<0,001 %
Rat c-peptide I	<0,03 %
Rat c-peptide II	<0,03 %
Mouse insulin	75%
Porcine insulin	476%
Sheep insulin	179%
Bovine insulin	78%

12.5 Conversion factor

1 µg corresponds to 174 pmol.

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	**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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