DRG® Insulin (Rat) Elisa (EIA-2048)

Revised 20 Apr. 2010 rm (Vers. 6.1) **For Veterinary Use**

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

INTENDED USE

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

PRINCIPLE OF THE PROCEDURE

The 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 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.

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.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 10, 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)

REAGENTS 1 X 96 KIT

Each 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 °C to 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 Colour coded yellow	1 vial	5 mL	Ready for use
Enzyme Conjugate 11 X (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 21 X 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 vial	22 mL	Ready for use
Stop Solution 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 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 °C to 8 °C for two months.

Preparation of Wash Buffer 1X solution

Prepare the needed volume of Wash Buffer by dilution of Wash Buffer 21 X in redistilled water 1 + 20 according to the table below. Mix gently.

Number of plates	*Wash Buffer 21 X*	*Redistilled water*
10 plates	2 bottles	8 000 ml
5 plates	180 ml	3600 ml
3 plates	110 ml	2200 ml
2 plates	70 ml	1400 ml
1 plate	35 ml	700 ml

Storage after dilution: 2 °C to 8 °C for 8 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 °C to 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 °C to 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 >5.5 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.

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) and wash buffer 1X solution.
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.

INTERNAL QUALITY CONTROL

Commercial controls such as DRG Diabetes Antigen Control Rat and Mouse, Low, Medium, and High (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 Calibrator 0, against the insulin concentration on a log-log or lin-log paper and construct a calibration curve.
2. Read the concentration of the unknown samples from the calibration curve.

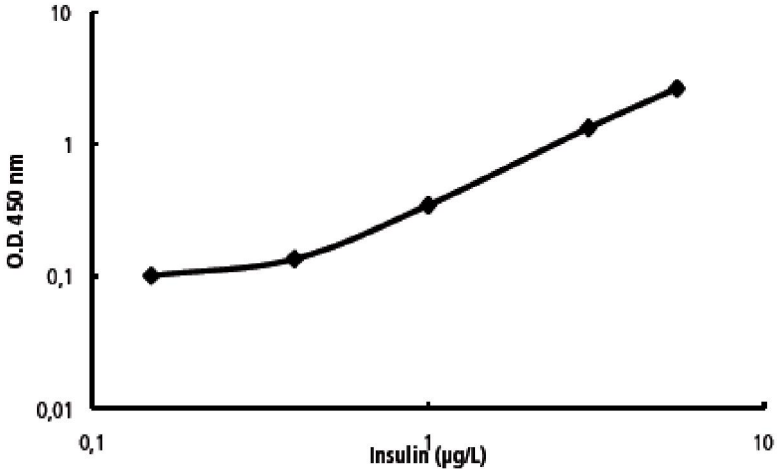
Example of results

Wells	Identity	A450	Mean conc. µg/L
1A–B	Calibrator 0	0,088/0,084	
1C–D	Calibrator 1*	0,097/0,105	
1E–F	Calibrator 2*	0,133/0,136	
1G–H	Calibrator 3*	0,351/0,338	
2A–B	Calibrator 4*	1,350/1,317	
2C–D	Calibrator 5*	2,627/2,668	
2E–F	Sample 1	0,162/0,157	0,49
2G–H	Sample 2	0,324/0,312	0,94
3A–B	Sample 3	1,304/1,202	2,9

*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

Performance limitations

Grossly lipemic, icteric or hemolysed samples do not interfere in the assay. Insulin is, however, degraded over time in heamolyzed 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 ≤0.15 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 80-93 % (mean 86 %).
Recovery upon dilution is 83-111 % (mean 96 %).

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 analysed in 4-replicates on 16 different occasions.

		Coefficient of variation		
Sample	**Mean value µg/L**	**within assay %**	**between assay %**	**total assay %**
1	0,47	5,1	10	11
2	0,96	3,1	4,4	4,7
3	2,8	2,8	3,2	3,5

SPECIFICITY

Human insulin	167%
Humanproinsulin	75%
Human C-peptide	< 0.05%
Insulin lispro (Humalog®)	167%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat C-peptide	< 0.001%
Rat proinsulin	7%
Mouse insulin	75%
Porcine Insulin	476%
Sheep Insulin	179%
Bovine Insulin	78%

CALIBRATION

The Rat Insulin ELISA is calibrated against an in-house reference preparation of rat insulin I.

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 or consequential.

REFERENCES

1. Korner J, Savontaus E, Chua SC, Jr., Leibel RL, Wardlaw SL (2001) Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. J Neuroendocrinol 13:959-966
2. Olsson R and Carlsson PO (2005) Better vascular engraftment and function in pancreatic islets transplanted without prior culture. Diabetologia 48:469-476
3. Rydtren T and Sandler S (2002) Efficacy of 1400 W, a novel inhibitor of inducible nitric oxide synthase, in preventing interleukin-1beta-induced suppression of pancreatic islet function in vitro and multiple low-dose streptozotocin-induced diabetes in vivo. Eur J Endocrinol 147:543-551

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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 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