DRG® Rat Insulin High Range (EIA-3985)

Revised 17 Feb. 2009 (Vers. 2.1) **Veterinary Use Only**

INTENDED USE

The High Range Rat Insulin ELISA provides a method for the quantitative determination of insulin in rat serum or plasma. This kit is intended for Veterinary Use Only.

PRINCIPLE OF THE PROCEDURE

The High Range 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.

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 >150 µg/l should be diluted 1/10 v/v with Standard 0.
Note! Buffer containing sodium azid (NaN_3) can not be used for sample dilution.

MATERIAL REQUIRED BUT NOT PROVIDED

- 10 µl micropipette with disposable tips
- Microplate reader with 450 nm filter
- Wash device for microtitration plates
- 50 µl and 200 µl repeating pipettes

- Tube (10-100 ml) for preparation of Conjugate
- Redistilled water
- 1000 ml/10 l bottle
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)

REAGENTS

Each High Range Rat Insulin ELISA kit contains reagents for 96 wells, sufficient for 42 samples and one Standard curve in duplicate on each plate. 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-well strips
For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.			
Standards 1, 2, 3, 4, 5	5 vials	500 µl	Ready for use
Concentration indicated on vial label. Color coded yellow.			
Standard 0	1 vial	5 ml	Ready for use
Color coded yellow			
Enzyme Conjugate 11X	1 vial	600 µl	Preparation, see below
(Peroxidase conjugated mouse monoclonal anti-insulin)			
Enzyme Conjugate Buffer	1 vial	6 ml	Ready for use
Color coded blue			
Wash Buffer 21X	1 bottle	40 ml	Dilute 1+20 with 800 ml redistilled water
Storage after dilution: 2–8°C for 4 weeks			to make Wash Buffer
Substrate TMB	1 vial	22 ml	Ready for use
(TMB) Colorless solution; Note! Light sensitive!			
Stop Solution	1 vial	7 ml	Ready for use
0.5 M H_2SO_4			

Preparation of enzyme conjugate

Prepare the needed volume of enzyme conjugate solution by dilution of Enzyme Conjugate 11X in Enzyme Conjugate Buffer (1+10) according to the table below.
When preparing enzyme conjugate 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 plate)	600 µl	6 ml
6 strips	300 µl	3 ml
4 strips	200 µl	2 ml

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

TEST PROCEDURE

Perform each determination in duplicate for Standards and unknowns. Prepare a standard curve for each assay run. Prepare enzyme conjugate solution and wash buffer.
All reagents must be brought to room temperature before use.

Add to anti-insulin wells	Standards	Unknowns
1. Standards	10 µl	–
2. Unknowns	–	10 µl
3. Enzyme conjugate solution	50 µl	50 µl

4. Incubate on a shaker (700-900 rpm) for 2 hours at room temperature (18–25°C).
5. Wash 6 times with automatic washer or
   Aspirate the reaction volume. Add 350 µl wash buffer to each well. Aspirate completely. Repeat 5 times.
   After final wash, invert and tap the plate firmly against absorbent paper.
6. Substrate TMB — 200 µl — 200 µl
7. Incubate for 15 minutes
8. Stop Solution — 50 µl — 50 µl
   Put the plate on the shaker for approximately 5 seconds to ensure mixing of Substrate and Stop Solution.
9. Measure the absorbance at 450 nm and evaluate.

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

INTERNAL QUALITY CONTROL

Commercial controls such as Insulin control, Mammalian (Code No. 10-1135-01) and/or internal serum pools with low, intermediate and high 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 insulin is obtained by computerized data reduction of the absorbance for the Standards, except for Standard 0, versus the concentration using cubic spline regression.

Manual Calculation

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

Example of results

Wells	Identity	Mean A_{450}	Mean conc. µg/l
1A-B	0 standard	0.070/0.072	
1C-D	3 µg/l standard	0.115/0.116	
1E-F	7.5 µg/l standard	0.194/0.200	
1G-H	30 µg/l standard	0.567/0.580	
2A-B	75 µg/l standard	1.310/1.321	
2C-D	150 µg/l standard	2.480/2.452	
2E-F	Unknown 1	0.601/0.618	32.2
2G-H	Unknown 2	0.330/0.331	15.3

CONVERSION FACTOR

1 µg corresponds to 0.174 nmol:

µg/l	3	7.5	30	75	150
nmol/l	0.52	1.30	5.22	13.05	26.1

LIMITATIONS OF THE PROCEDURE

Performance limitations

Grossly lipemic, icteric or hemolysed samples do not interfere in the assay.

EXPECTED VALUES

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

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit is 1.5 µg/l calculated as two standard deviations above the zero standard.

Recovery

Recovery upon addition is 106 %
Recovery upon dilution is 100 %.

Hook effect

Samples with a concentration of up to at least 576 µg/l can be measured without giving falsely low results.

Precision

Each sample was analysed in 4-replicates on five different occasions.

Sample µg/l	Mean value	Coefficient of variation		
	µg/l	within assay %	between assay %	total assay %
1	32.7	4.8	2.5	5.4
2	16.2	4.5	4.1	6.1

Specificity

	%
Human insulin	167
Human proinsulin	75
Human C-peptide	<0,05
Insulin Lispro (Humanlog®)	167
IGF-I	<0,02
IGF-II	<0,02
Rat C-peptide	<0,001
Rat Insulin	100
Rat proinsulin	7
Mouse Insulin	75
Porcine Insulin	476
Sheep Insulin	179
Bovine Insulin	78

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

Kullin M, Li Z, Bondo Hansen J, Welsh N, Karlsson FA, Sandler S. (2003). Protection of rat pancreatic islets by potassium channel openers against alloxan, sodium nitroprusside and interleukin-1beta mediated suppression-possible involvement of the mitochondrial membrane potential.
Diabetologia 46:80-88

von Mach MA, Schlosser J, Weiland M, Feilen PJ, Ringel M, Hengstler JG, Weilemann LS, Beyer J, Kann P, Weber M, Schneider S. (2003). Cryopreservation of islets of Langerhans: Optimization of protocols using rat pancreatic tissue.
EXCLI Journal 2:6-21

SUMMARY PROTOCOL SHEET

DRG High range Rat Insulin ELISA

Add standards and samples	10 µl
Add Conjugate solution	50 µl
Incubate	2 hours at 18-25°C on a plate shaker
Wash	6 times
Add Substrate	200 µl
Incubate	15 minutes
Add Stop solution	50 µl (Shake for 5 seconds to ensure mixing)
Measure A450	