DRG® Insulin ELISA (EIA-1825)

CE

Revised 1 Apr. 2010 rm (Vers. 6.0) **IVD**

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

1 INTENDED USE

Insulin ELISA provides a method for the quantitative determination of human insulinin serum or plasma.

2 SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the ß-cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain.
Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. Its principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, epinephrine (adrenaline), growth hormone and cortisol.
Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

3 PRINCIPLE OF THE PROCEDURE

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 (TMB).The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

4 WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- The contents 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 H2SO4. Follow routine precautions for handling hazardous chemicals.
- All patient specimens should be handled as of capable of transmitting infections.

5 MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100, 200 and 1000 µl (repeat pipettes preferred for addition of enzyme conjugate solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water

- Microplate reader (450 nm filter)
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device

6 REAGENTS

Each 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 wells completely reseal the bag using adhesive tape and use within two months.			
Calibrators 1, 2, 3, 4, 5 Concentration indicated on vial label (recombinant human insulin) Color coded yellow	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	1.2 ml	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial 1	2 ml	Ready for use
Wash Buffer 21X Dilute with 1000 ml redistilled water to make wash solution Storage after dilution: 2–8°C for 4 weeks.	1 bottle	50 ml	
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

6.1 Preparation of enzyme conjugate 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 solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently. Use within one day.

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Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µl	7.0 ml
6 strips	500 µl	5.0 ml
4 strips	350 µl	3.5 ml

7 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.

7.1 Preparation of samples

No dilution is normally required, however, samples containing > 200 mU/l should be diluted 1+9 v/v with Calibrator 0.

8 TEST PROCEDURE

All reagents and samples must be brought to room temperature before use.
Prepare a calibration curve for each assay run.

1. Prepare enzyme conjugate solution and wash buffer.
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 to each well.
5. Incubate on a plate shaker (700-900 rpm) for 1 hour at room temperature (18–25°C)
6. Wash 6 times with Wash 6 times with 700 µl 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 for 15 minutes at room temperature (18–25°C)
9. Add 50 µl Stop Solution to each well.
   Place plate on a 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.

9 INTERNAL QUALITY CONTROL

Commercial controls 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, Calibrators and controls.

10 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 calibration curve.
2. Read the concentration of the unknown samples from the calibration curve.

Example of results

Wells	Identity	A_{450}	Mean conc. mU/l
1 A–B	Calibrator 0	0.070/0.071	
1 C–D	Calibrator 1 *	0.105/0.106	
1 E–F	Calibrator 2 *	0.202/0.204	
1 G–H	Calibrator 3 *	0.434/0.470	
2 A–B	Calibrator 4 *	1.348/1.351	
2 C–D	Calibrator 5 *	2.451/2.476	
2 E–F	Unknown 1	0.222/0.214	11.1
2 G–H	Unknown 2	0.546/0.538	35.6
3 A–B	Unknown 3	1.941/1.978	153

* Concentration indicated on the vial label.

Conversion factor

1 µg/l = 29 mU/l; 1mU/l = 6.0 pmol/l

11 LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.
Application of this test to individuals already undergoing insulin therapy is complicated by formation of anti-insulin antibodies that are capable of interfering in the assay.
Grossly lipemic, icteric or hemolysed samples do not interfere in the assay.

12 EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own.

Fasting levels for 137 tested, apparently healthy individuals, yielded a mean of 9.2 mU/l, a median of 6.9 mU/l and a range, corresponding to the central 95% of the observations, of 2–25 mU/l.

13 PERFORMANCE CHARACTERISTICS

13.1 Detection limit

The detection limit is 1 mU/l calculated as two standard deviations above the Calibrator 0.

13.2 Recovery

Recovery upon addition is 94–113% (mean 104%).

13.3 Hook effect

Samples with a concentration of up to 30 000 mU/l can be measured without giving falsely low results.

13.4 Precision

Each sample was analysed in 6-replicates on six different occasions.

Sample	Mean value mU/l	Coefficient of variation		
		Within assay %	Between assay %	Total assay %
1	11	3.4	3.6	5.0
2	36	4.0	2.6	4.7
3	80	2.8	2.8	4.0
4	154	3.2	2.9	4.4

13.5 Specificity

The following cross reactions have been found:

C-peptide	< 0.01%
Proinsulin	< 0.01%
Proinsulin des (31–32)	< 0.5%
Proinsulin split (32–33)	< 0.5%
Proinsulin des (64–65)	98%
Proinsulin split (65–66)	56%
Insulin lispro (Humalog®, Eli Lilly)	< 0.000003%
Insulin aspart	< 3.2%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	0.7%
Mouse insulin	0.3%
Porcine insulin	374%
Sheep insulin	48%
Bovine insulin	31%

14 CALIBRATION

The Insulin ELISA kit is calibrated against 1st International Reference Preparation 66/304.

15 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.

16 REFERENCES

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