DRG® Iso-Insulin (EIA-2336)

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IVD

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

INTENDED USE

DRG Iso-Insulin ELISA provides a method for the quantitative determination of insulin in human serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthezised in the β-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.

PRINCIPLE OF THE PROCEDURE

Iso-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 anti-genic determinants on the insulin molecule. During incubation insulin in the sample react with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to 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

- 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 H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All patient specimens should be handled as of capable of transmitting infections.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50 and 200 µl (repeat pipettes preferred for addition of Enzyme Conjugate, Substrate TMB and Stop Solution).

- Beakers and cylinders for reagent preparation
- Redistilled water
- Microplate reader with 450 nm filter
- Plate shaker (The recommended velocity is 700–900 cycles per minute, orbital movement)
- Microplate washing device

REAGENTS

Each Iso-Insulin ELISA kit contains reagents for 96 wells, sufficient for 43 samples and one calibration 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)** **For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.**	**1 plate 8-well strips**	**96 wells**	**Ready for use**
Calibrators 1, 2, 3, 4 Concentration indicated on vial label (recombinant human insulin)	4 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	600 µl	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	6 ml	Ready for use
Wash Buffer 21X	1 bottle	40 ml	Dilute with 800 ml redistilled water to make Wash Buffer Storage after dilution: +2–8°C for 4 weeks.
Substrate TMB (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 solution

Prepare the needed volume of enzyme conjugate by dilution of Enzyme Conjugate 11X, (1+10) in Enzyme Conjugate Buffer or according to the table below. Mix gently.

When preparing enzyme conjugate solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12	1 vial	1 vial
8	350 µL	3.5 mL
6	250 µL	2.5 mL
4	200 µL	2.0 mL

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

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

Samples containing >100 mU/l should be diluted e.g. 1/10 v/v with Calibrator 0.

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 solution.
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. Pipette 50 µl Enzyme Conjugate solution to each well.
5. Incubate on a plate shaker for 1 hour (700 – 900 rpm) 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.
8. Incubate for 15 minutes at room temperature (18–25°C).
9. Add 50 µl Stop Solution.
   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.

INTERNAL QUALITY CONTROL

Commercial controls such as Diabetes antigen control (Code No 10-1134-01/10-1164-01) 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 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 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. mU/l
1A–B	Calibrator 0	0.066/0.067	
1 C–D	Calibrator 1 *	0.084/0.087	
1E–F	Calibrator 2 *	0.161/0.165	
1G–H	Calibrator 3 *	0.595/0.599	
2A–B	Calibrator 4 *	2.377/2.347	
2C–D	Unknown 1	0.270/0.272	16.4
2E–F	Unknown 2	1.146/1.192	53.6
2G–H	Unknown 3	2.044/2.150	92.7

- Concentration indicated on the vial label.

Conversion factor
1 µg/l = 29 mU/l; 1 mU/l = 6.0 pmol/l

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 hemolyzed samples do not interfere in the assay.

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.
Comparison studies between DRG Iso-Insulin ELISA and DRG Insulin ELISA, have been performed with 90 samples. The values found show a good correlation between the two techniques, r = 0.98.
Thus, the expected values for DRG Insulin ELISA can be used for DRG Iso-Insulin ELISA as well.

Mean fasting levels for 137 tested, apparently healthy individuals, were 10 mU/l, a median of 7 mU/l and a range-corresponding to the central 95% of the observations – of 2–25 mU/l.

PERFORMANCE CHARACTERISTICS

Detection limit

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

Recovery

Recovery upon addition is 101%.

Hook effect

Samples with a concentration of up to at least 2000 mU/l can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4-replicates on eight different occasions.

		Coefficient of variation		
Sample	**Mean value mU/l**	**within assay %**	**between assay %**	**total assay %**
1	15.9	3.0	3.9	4.9
2	53.2	2.8	3.0	4.1
3	90.9	3.2	3.0	4.4

Specificity

Insulin	100%
Insulin lispro (Humalog®, Eli Lily)	89%
Insulin aspart	80%
Insulin detemir	22%
Insulin glargin	44%
Insulin glulisine	100%
C-peptide	< 0.1%
Proinsulin	54%
Proinsulin des (31-32)	58%
Proinsulin split (32-33)	56%
Proinsulin des (64-65)	66%
Proinsulin split (65-66)	78%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	71%
Mouse insulin	49%
Porcine insulin	306%
Sheep insulin	131%
Bovine insulin	58%

CALIBRATION

DRG Iso-Insulin ELISA kit is calibrated against 1st International Reference Preparation 66/304 for human insulin.

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.
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