Dako

Insulin ELISA Kit

Instructions for Use

REAGENTS FOR 96 DETERMINATIONS

For *in vitro* diagnostic use

IVD

CE

Manufactured by

Mercodia AB
Sylveniusgatan 8A
SE-754 50 Uppsala
Sweden

EXPLANATION OF SYMBOLS USED ON LABELS

	Reagents for 96 determinations
	Expiry date
	Store between 2-8°C
	Lot No.
	For *in vitro* diagnostic use

	GHS hazard pictogram (consult warnings and precautions section)
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INTENDED USE

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

As with all diagnostic tests, a definitve 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. The analysis should be performed by trained laboratory professionals.

SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the beta-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 elevated in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

PRINCIPLE OF THE PROCEDURE

Insulin ELISA Kit 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 microplate welles. 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.

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 Calibrators 0, 1, 2, 3, 4 and 5 contain <0.06% reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one. The calibrators are labeled:

H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

- The Enzyme Conjugate Buffer contains <0.06% reaction mass of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one. The Enzyme Conjugate Buffer is labeled:

H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

- The Stop Solution in this kit contains 0.5 M sulfuric acid, H_2SO_4 and is labeled:

H318 - Causes serious eye damage.
H315 – Causes skin irritation.
P280 – Wear protective gloves. Wear eye or face protection.
P264 – Wash hands thoroughly after handling.
P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.
P332 + P313 – If skin irritation occurs: Get medical attention.
P305 + P351 + P338 P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

- All samples should be handled as of capable of transmitting infections.
- Each well can only be used once.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes with appropriate volumes (multipipettes preferred for addition of enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader with 450 nm filter
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

REAGENTS 1 X 96

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 2 months.			
Calibrators 1, 2, 3, 4, 5 Recombinant human insulin Color coded yellow Concentration indicated on vial label	5 vials	1 mL	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	12 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 2 months	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution
Substrate TMB Colorless solution *Note! Light sensitive!*	1 bottle	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 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. Use within 1 day.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µL	7.0 mL
4 strips	350 µL	3.5 mL

 Storage after dilution: 2 - 8°C

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 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.

Preparation of samples

No dilution is normally required for serum and plasma samples, however, samples with a concentration above Calibrator 5 should be diluted in Calibrator 0.

TEST PROCEDURE

All reagents and samples must be brought to room temperature (18-25°C) before use.
Prepare a calibrator curve for each assay run.

1. Prepare enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accomodate calibrators, controls and samples in duplicate.
3. Pipette 25 µL each of calibrators, controls and samples into appropriate wells.
4. Add 100 µL of enzyme conjugate 1X solution 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 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 buffer 1X 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.
7. Add 200 µL Substrate TMB into each well.
8. Incubate on the bench 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 (OD) at 450 nm and calculate results.
    Read within 30 minutes.

Note! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution.

INTERNAL QUALITY CONTROL

Commercial controls and/or internal serum pools with low, intermediate and high insulin concentration 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, preparation date of components, OD values for the blank, calibrators and controls.
Laboratories should follow government regulations or accreditation requirements for quality control frequency.

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 calibrator curve.
2. Read the concentration of the samples from the calibrator curve.

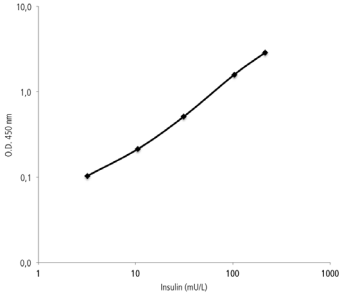
Example of results

Wells	Identity	A_{450}	Mean Conc. mU/L
1 A-B	Calibrator 0	0.052/0.051	
2 C-D	Calibrator 1*	0.104/0.103	
3 E-F	Calibrator 2*	0.215/0.212	
4 G-H	Calibrator 3*	0.521/0.502	
2 A-B	Calibrator 4*	1.599/1.574	
2 C-D	Calibrator 5*	2.861/2.902	
2 E-F	Sample 1	0.182/0.179	8.3
2 G-H	Sample 2	0.462/0.475	28.1
3 A-B	Sample 3	1.187/1.219	77.4

*Concentration stated on vial label

Example of calibrator curve

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



Conversion factor

1 µg/L = 23 mU/L; 1 mU/L = 6.0 pmol/L; 1 mU/L = 1 µIU/mL

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. However, hemolysis in serum and plasma samples may result in a degradation of insulin which could give falsely low values and contributes to higher inter assay variation. The degradation is dependent on time, temperature and the hemoglobin concentration. Keep hemolyzed samples cold or on ice to prevent the insulin degradation.

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.

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 1 mU/L as determined by the methodology described in ISO11843-Part 4.

Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition is 94–113% (mean 104%).
Recovery upon dilution is 101-110% (mean 106%).

Hook effect

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

Precision

Each sample was analyzed in 6 replicates on 6 different occasions.

Sample	Mean value	Coefficient of variation		
	mU/L	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

Specificity

The following crossreactions 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 aspart	4 %
Insulin detemir	n.d.
Insulin glargine	24 %
Insulin glulisine	n.d.
Insulin lispro	n.d.
IGF-I	< 0.02 %
IGF-II	< 0.02 %
Rat insulin	0.7 %
Mouse insulin	0.3 %
Porcine insulin	374 %
Ovine insulin	48 %
Bovine insulin	31 %

n.d.=not detected

CALIBRATION

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

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure may affect the results, in which event Dako disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Dako and its authorised distributors, in such event, shall not be liable for damages indirect or consequential.

REFERENCES

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SUMMARY OF PROTOCOL SHEET
Insulin ELISA Kit

Add calibrators, controls* and samples	25 µL
Add enzyme conjugate 1X solution	100 µL
Incubate	1 hour at 18-25°C on a plate shaker 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add Substrate TMB	200 µL
Incubate	15 minutes (18-25°C)
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A_{450}	Evaluate results

*not provided

For full details see page 9