RK-400CTACE230324

INSULIN [I-125] IRMA KIT

(REF: RK-400CT)

The insulin [I-125] assay system provides the quantitative determination of insulin in human serum. Insulin can be assayed in the range of 1.8-500 µIU/mL (0.06-17.5 ng/mL). Each kit contains materials sufficient for 100 assay tubes, permitting the construction of one standard curve and assay of 42 unknowns and 1 control in duplicate.

Introduction

The insulin is a light polypeptide hormone with molecular weight 6000. It is synthesized in the beta cells of the pancreas from the precursor proinsulin. Proinsulin enzymatically splits into insulin and C-peptide that are stored in the pancreas and release from there in equimolar quantities into the blood system. Insulin consists of two polypeptide chains: A (21 amino acids) and B (30 amino acids) connected to each other by two disulphide bridges. While in the amino acid sequence of C-peptide great differences can be observed in the case of various mammals, in insulin these differences are insignificant.

Insulin is an important metabolic hormone that has several direct and indirect effects on the organism. Its general influence is that it stimulates the synthesis and accumulation of macromolecules playing role in energy supply and in the regulation of metabolic processes. Insulin increases the rate of glucose transport trough the cell membranes, helps the admission of other monosaccharides, amino acids, potassium and magnesium ions into the cells. Insulin promotes the utilization and oxidation of glucose, glycogenesis, lipogenesis, as well as the formation of ATP, DNA and RNA.

The symptoms of diabetes mellitus can be attributed to the inappropriate insulin response to glucose concentration. While in the case of unambiguous diabetes reduced insulin response is observed, in various early stages of diabetes the insulin level of the patients may be normal or even high, and increase of various degrees can be found in stimulation tests. The fasting hyperglycaemia of not overweight patients is usually accompanied by normal circulatory insulin level, while in obese patients this level is high, in proportion of overweight.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system. The ^{125}I labelled signal-antibody binds to an epitope of the Insulin molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich". During a 2-hour incubation period immuno-complex is immobilized to the reactive surface of streptavidin-coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter. The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of Insulin, the unknown concentration of Insulin in patient samples can determined.

Contents of the kit

1. 1 bottle of TRACER, Ready to use. 21 mL per vial, containing < 980 kBq ^{125}I-signal and capture antibody in buffer with red dye and 0.1 % NaN_3.

2. 6 vials of STANDARDS (S0-S5), lyophilized. 1.0 mL each, in equine serum with 0.1 % NaN_3. App. (S0-S5): 0, 5, 15, 50, 150, 500 µIU/mL. The exact concentrations are indicated on each vial and in the quality certificate enclosed.

3. 1 vial of CONTROL SERUM, lyophilized. 1.0 mL, in human serum with 0.1 % NaN_3. The concentration of control serum is specified in the quality certificate enclosed.

4. 2 boxes of COATED TUBES, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

5. 1 bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN_3. See *Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

Round bottom polystyrene or polypropylene assay tubes, about 12 x 75 mm, precision pipettes (50 µl, 100 µl and 500 µl), vortex mixer, orbital shaker, gamma counter

Recommended tools and equipment

repeating pipette

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 48 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, haemolyzed or turbid specimens.

Preparation of reagents, storage

Add 1.0 mL distilled water to the *lyophilised standard and control serum*, and mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes. For repeated use the rest of reagent can be stored at -20 °C for two months.

Add the wash buffer concentrate (20 mL) to 1000 mL distilled water to obtain 1020 mL wash solution. Upon dilution store at 2-8°C until expiration.

Store the reagents between 2-8°C after opening. At this temperature each reagent (except reconstituted standard and control) is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Label coated tubes in duplicate for each standard, control serum & samples.
2. Homogenize all reagents & samples by gentle mixing to avoid foaming.
3. Pipette 100 µl of standards, control & samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
4. Pipette 200 µl of tracer into each tube. (Set aside 2 tubes for total counts.)
5. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed so that liquid is constantly rotating or shaking in each tube. (200 - 600 rpm recommended)
6. Incubate tubes for 2 hours, shaking at room temperature.
7. Add 2.0 mL of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside-down position place the rack on an absorbent paper for 2 minutes.
8. Return the tube-rack to an upright position, and repeat step-7 two more times.
9. Count each tube for at least 60 seconds in a gamma counter.
10. Calculate the Insulin concentrations.

Table 1. Assay Protocol, Pipetting Guide
(all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**100**		
Control			**100**	
Sample				**100**
Tracer	**(200)**	**200**	**200**	**200**
Shake for 2 hours at room temperature				
Wash b.		**2000**	**2000**	**2000**
Decant the fluid & blot on filter paper				
Wash b.		**2000**	**2000**	**2000**
Decant the fluid & blot on filter paper				
Wash b.		**2000**	**2000**	**2000**
Decant the fluid & blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (cpm) for each pair of assay tubes. Calculate the normalized percent binding for each standard, control & sample respectively by using the following equation:

$$B/T\ (\%) = \frac{S_{1\text{-}5} / C / M_x\ (cpm) - S0\ (cpm)}{T(cpm)} \times 100$$

Using logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of Insulin.

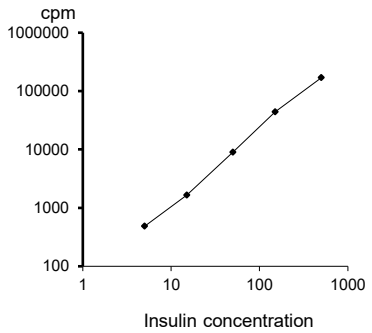
Determine the Insulin concentration of the control & unknown samples by interpolation from the standard curve.

Automated data processing systems are also applicable.

Typical assay data

Tubes	Mean cpm	B/T%	µIU/mL
T	381475		
S0	124	0	
S1	484	0.1	
S2	1650	0.4	
S3	8988	2.4	
S4	44294	11.6	
S5	169191	44.4	
C	6678		40.1

Typical standard curve



Characterization of assay

Calibration

Standards are calibrated against the international reference standard NIBSC 66/304.

Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17.

Limit of Blank (LoB): 0.82 µIU/mL
Limit of Detection (LoD): 1.23 µIU/mL
Limit of Quantitation (LoQ): 1.8 µIU/mL

Specificity

The cross-reactivity with human Proinsulin is < 0.5% and there is no C-Peptide interference for concentrations up to 986 ng/mL. Cross-reactivity with insulin analogues is undetectable for Degludec, Lispro, Glulisine, Aspart and Detemir. Cross-reaction with Glargine is 9.9%.

Intra-assay precision

5 patient samples were assayed in 15 replicates to determine intra-assay precision. Values obtained are shown below.

Sample	mean µIU/mL	SD	CV %
1	5.41	0.23	4.2
2	13.83	0.33	2.4
3	45.10	0.36	0.8
4	89.43	3.93	4.4
5	149.2	1.19	0.8

Inter-assay precision

To determine inter-assay precision 6 patient samples were measured in duplicates in 15 independent assays by 2 operators using different kit batches. Values obtained are shown below.

Sample	mean µIU/mL	SD	CV %
1	4.7	0.77	16.5
2	20.9	0.90	4.3
3	40.4	0.95	2.3
4	65.0	1.45	2.2
5	102.2	3.07	3.0
6	152.6	4.86	3.2

Multisite precision

Multisite precision was calculated using 5 serum pools at different Insulin concentrations, according to CLSI document EP05-A3. Results: Repeatability < 5%, Within laboratory precision < 6%, Reproducibility < 8%, for all concentrations evaluated.

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of Insulin. The average recovery for 6 serum samples spiked with Insulin at 3 levels each was 95.1% (80.6% – 101.5%).

Linearity

32 individual serum samples were diluted two-fold with zero-standard and measured according to kit protocol. Mean dilution recovery was 90.2% (81.7% - 105.3%). The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$Y = 0.9182X - 0.5741 \quad R^2 = 0.9933$

Interference

Samples containing up 262 µmol/L bilirubin, 32.1 mmol/L triglycerides, 4.7 g/L haemoglobin and 100 ng/mL biotin do not affect the concentration of Insulin assayed.

Reference Interval

It is highly recommended that each laboratory determines its own normal range. Fasting patients with normal blood glucose and presumably healthy blood donors were evaluated. Values presented below should be only considered as a guide.

	No. of samples	Median (µIU/mL)	95% central range (µIU/mL)
Fasting	100	5.15	1 – 30
Postpandrial	120	14.15	3 - 67

The results obtained should only be interpreted in the context of the overall clinical picture. None of in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

Conversion of SI units can be performed according to the following formula**:**
1 µIU/mL = 5.99 pmol/l
1 ng/mL = 28.7 µIU/mL

Limitations

• The reagents supplied in this kit are optimized to measure Insulin levels in serum.
• Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
• Haemolyzed and lipemic specimens may give false values and should not be used.
• The KIT has no "high-dose hook" effect with Insulin levels up to 2500 µIU/mL. Samples expected to have concentrations greater than the highest standard should be diluted with the S_0 (0 µIU/ml) and reassayed.
• The results of this assay should be used in conjunction with other pertinent clinical information.

Procedural notes

Source of error! To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.
Addition of wash buffer. For the addition of wash buffer, the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.
Additional information: Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions and warnings

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials.*

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 68 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 67 days from availability.

	Used by	LOT	Batch code
2 8°	Temperature limitation	CONTROL	Control
	Caution, consult accompanying documents	CAL	Standard
	Biological risks	TRAC	Tracer
	Consult instructions for use	WASHB	Wash buffer
REF	Catalogue number	CT	**Coated tube**
	Manufacturer		Radioactive material
IVD	*In vitro* diagnostic medical device		CE

WEB site: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

IZOTOP
H-1121 Budapest, Konkoly Thege M. út 29-33.
✉ H-1535 Budapest, P.O.B. 851 Hungary
Phone: + 36 1 3959081
Fax: + 36 1 395924

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