rInsulin [^{125}I] IRMA KIT

(REF: RK-547CT)

For Research Use Only. Not for use in diagnostic procedures.

The rInsulin IRMA system provides direct quantitative *in vitro* determination of rat insulin in plasma and serum. Rat insulin can be measured in the range 0.3-30 ng/mL. Each kit contains materials sufficient for 100 determinations permitting the construction of one standard curve and the assay of 44 unknowns in duplicate.

Introduction

Rat insulin is a pancreatic hormone whose molecular weight is about 6000. It is a protein composed of two polypeptide chains, a shorter A-chain of twenty-one residues and a longer B-chain of thirty. The two chains are connected by two disulphide (-S-S-) linkages, while a third such linkage forms an intra-chain precursor called pro-insulin , in which the future A- and B-chains are linked end to end by a peptide strand, C-peptide, before being joined by their –S-S-bonds. It is found in the ß-cell granules in the pancreatic Islets of Langerhans. Specific proteases act on pro-insulin to release the C-peptide and insulin within the granule. On stimulation the C-peptide and insulin are released into the bloodstream in approximately equimolar amounts.

Rat insulin differs from most other species in that it has two forms that are products of non-allelic genes. Translation of the two insulin mRNAs results in the synthesis of two preproinsulins differing by 7 amino acids. Processing of these peptides involves removal of the pre region and formation of proinsulins differing in 4 of 86 amino acids. The proinsulins are cleaved to mature insulins 1 and 2 which have identical A chains but differ by 2 amino acids in the B chain (positions 9 and 29). They are found roughly in the proportion 60% insulin 1 and 40% insulin 2 in the pancreas.

Several factors can effect the release of insulin. One of the main regulators of insulin release is the amount of glucose in the blood. A rise in blood glucose stimulates the release of insulin while a fall in blood glucose suppresses its secretion. Amino acids also stimulate insulin-release to allow their uptake into muscle cells. Insulin is considered to be an anabolic hormone in that it promotes the synthesis of protein, lipid and glycogen and it inhibits the degradation of these compounds. The key target tissues of insulin are liver, muscle and adipose tissue. In promotes cell growth in many different cell types and is an absolute requirement for normal growth in all immature animals. Insulin exerts its effect through a receptor complex comprising two a sub-units of molecular weight 135 kDa and two ß sub-units of molecular weight 90 kDa. It is also well known for its involvement in diabetes, where insulin deficiency results in aberrant blood glucose homeostasis.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunometric assay system. This assay is based on a two-step procedure. In the first step the standards and samples are incubated in streptavidin coated tubes with biotin labelled monoclonal antibody (capture antibody). During a 1-hour incubation period with continuous agitation the capture antibody - antigen complex is developed and immobilized on the reactive surface of tubes. After incubation tubes are washed repeatedly. In the second step the [^{125}I] labelled monoclonal antibody (signal antibody) is added. It binds to an epitope of the insulin molecule different from that recognised by the capture-antibody, developing the formation of a capture antibody - antigen - signal antibody complex, also referred to as a 'sandwich'. After the one-hour-incubation period with continuous agitation the reaction mixture is washed repeatedly, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in the tubes. The unknown concentration of rat insulin in samples is read off a calibration curve constructed by plotting binding values against a series of calibrators containing known amount of rat insulin.

Contents of the kit

1. 1 bottle TRACER (6 mL), ready to use, containing about 740 kBq ^{125}I-anti-rat Insulin in buffer with red dye 0.1 % NaN_3.
Store at 2-8 °C.

2. 6 vials STANDARDs (S0-S5), (S0 2 mL, S1-S5 0.5 mL per vial), ready to use, containing 0.3 (S1); 1 (S2); 3 (S3); 10 (S4); 30 (S5) ng/mL rat Insulin in buffer with yellow dye and 0.1 % sodium azide.
Store at 2-8 °C.

3. 1 bottle ANTISERUM (6 mL), ready to use, containing biotin labelled anti-rat insulin in buffer with blue dye and 0.1 % sodium azide.
Store at 2-8 °C.

4. 2 boxes COATED TUBES, ready to use.
2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.
Store at 2-8 °C.

5. 2 bottles WASH BUFFER CONCENTRATE (20 mL per bottle), with 0.2 % sodium azide.
Store at 2-8 °C.

See *Preparation of reagents.*

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, adsorbent tissue, gamma counter.

Recommended tools and equipment

Repeating pipettes (e.g. Eppendorf or else), dispenser with 2-L reservoir (instead of the 2-ml pipette).

Specimen collection

This section is provided for guidance only. It remains the investigator's responsibility to validate the chosen sample collection technique.

Serum samples

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying.

Plasma samples

It is advised that if measurements are to be made on plasma samples, blood should be collected into tubes containing EDTA. Blood should be centrifuged immediately to remove cells and the plasma stored below -15°C prior to analysis.

Samples may need to be diluted depending on the expected concentration. The zero standard, S0 may be used for this purpose.

Preparation of reagents, storage

Store the reagents between 2-8°C after opening. At this temperature each reagent is stable until the expiration date of the kit. The actual expiration date is given on the package label.

Preparation: Equilibrate all reagents and samples to room temperature prior to use.

Wash buffer: Add the wash buffer concentrates (2 x 20 mL) to 1400 mL distilled water to obtain 1440 mL wash solution. Upon dilution store at 2-8°C until the expiration date of the kit.

Assay procedure

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

1. Equilibrate reagents and samples to room temperature before use.
2. Prepare reagents as described in the previous section.
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Label the tubes for duplicates of each standard (S0-S5), and sample (SX). Label two non-coated test tubes for total count (T).
5. Pipette 50 µL each of standards and samples into the appropriate tubes.
6. Pipette 50 µL antiserum into each tube.
7. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
8. Incubate tubes for 1 hour, shaking at room temperature.
9. 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.
10. Return the tube-rack to an upright position and repeat step-8 2 times
11. Pipette 50 µL of tracer into each tube.
12. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
13. Incubate tubes for 1 hour, shaking at room temperature.

14. 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.
15. Return the tube-rack to an upright position and repeat step-8 2 times.
16. Count each tube for at least 60 seconds in a gamma counter.
17. Calculate the rat insulin concentrations of the samples as described in calculation of results or use special software.

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

Tubes	Standard	Sample
Standard	**50**	
Sample		**50**
Antiserum	**50**	**50**
Shake for 1 hour at room temperature		
Wash buffer	**2000**	**2000**
Decant the fluid and blot on filter paper		
Repeat the washing step 2 times		
Tracer	**50**	**50**
Shake for 1 hour at room temperature		
Decant the fluid and blot on filter paper		
Wash buffer	**2000**	**2000**
Decant the fluid and blot on filter paper		
Repeat the washing step 2 times		
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 and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}5} / M_x\ (cpm) - S_0\ (cpm)}{T(cpm)} \times 100$$

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

Determine the rat Insulin concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	Conc. ng/ml	Count cpm	Mean cpm	B/T%
T		259835 259065	259450	
S0	0	422 457	440	0.16
S1	0.3	1049 958	1004	0.22
S2	1	3011 2943	2977	0.97
S3	3	10839 11918	11379	4.22
S4	10	49705 50570	50138	19.16
S5	30	135867 137854	136861	52.58

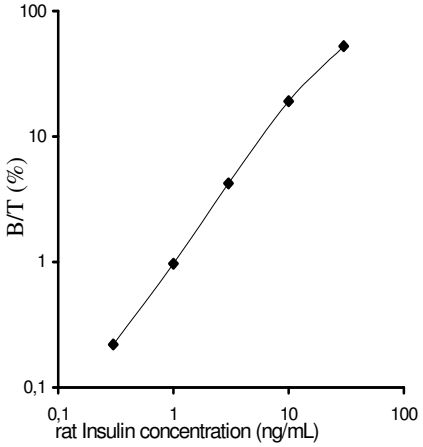


Figure1: A typical standard curve
(*Do not use to calculate unknown samples)*

Characterization of assay

Stability

The components of this assay system will have a shelf-life of at least 3 weeks from the date of despatch.
Upon arrival, all components should be stored at 2-8°C where they are stable until the expiry date printed on the end pack label.

Sensitivity

The analytical sensitivity or minimum detectable limit is calculated by the interpolation of the mean counts of zero standard plus 2 standard deviation from the standard curve. Determination was carried out using 15 replicates of zero standard response.
The value of analytical sensitivity is 0.1 ng/mL.

Procedural notes

1) Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) 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.

3) The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

4) Do not leave the cap off of the storage bottle for prolonged periods of time.

Limitations

• Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
• Hemolyzed and lipemic specimens may give false values and should not be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions

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.

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 57 mg.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

Safety data sheet

Product name:
Sodium azide
CAS No. 26628-22-8
R: 22-32 Toxic if swallowed. Contact with acids liberates very toxic gas.
S: (1/2)-28-45 (Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Composition:
Sodium azide solution.

Hazards identification:
Toxic if swallowed, inhaled, or absorbed through skin. May cause eye and skin irritation.

First aid measures:
In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.

Fire fighting measures:
Dry chemical powder. Do not use water.

Accidental release:
Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick-up is complete.

Handling and storage:
Wear suitable protective clothing including overalls, safety glasses and gloves. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling.

Personal protection:
See above instructions for handling and storage.

Physical and chemical properties:
Formula weight: 65.01. Density: 1.850.

Stability and reactivity:
Avoid contact with metals and acid chlorides. This yields a very toxic gas.

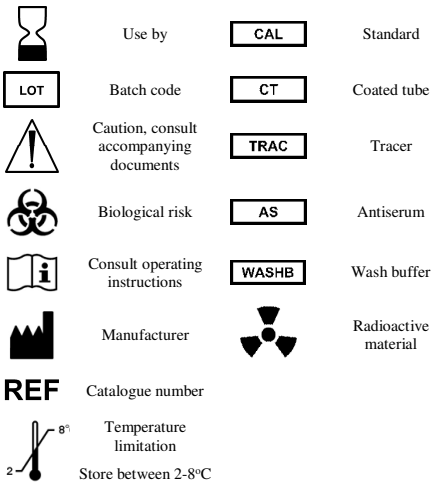
Toxicological information:
LD50: 27 mg/kg oral, rat
LD50: 20 mg/kg skin, rabbit

Ecological information:
Not applicable

Disposal consideration:
Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.
Note: Inorganic azides will react with lead and copper plumbing fixtures to give explosive residues. Disposal of significant quantities of azides via such plumbing is not recommended.

Transport information :
No special considerations applicable.

Regulatory information:
The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.



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