RK-50PACE200901

IAA [I-125] RIA KIT

Catalogue numbers:
RK-50P50, kit for 50 determinations
RK-50P100, kit for 100 determinations

The IAA [I-125] RIA system provides a direct quantitative determination of autoantibodies to Insulin in human serum. IAA can be assayed in the range of 0-50 U/mL. Each kit contains materials sufficient for 100 assay tubes permitting the construction of one standard curve and the assay of 43 unknowns in duplicate.

Introduction

Type 1 diabetes mellitus (T1DM) results from a chronic autoimmune destruction of insulin-secreting pancreatic beta cells. During the preclinical phase, this process is characterised by the formation of autoantibodies to beta cell antigens, which can be detected years before clinical symptoms. Circulating autoantibodies to pancreatic beta cells are important serological markers of type 1 diabetes mellitus. The antigens recognised by these antibodies include Insulin (IAA), Glutamic Acid Decarboxylase (GAD65) and Protein Tyrosine Phosphatase (IA2).
The prevalence of IAA is significantly elevated in subjects developing the disease in childhood and are often the first autoantibodies to be detected before the onset of the disease.
The combination of tests for GAD65, IA2 and IAA forms the basis of current strategies for predicting the future onset of type 1 diabetes.
The risk of type 1 diabetes increases as the number of relevant autoantibodies detected increases.

Principle of method

This IAA kit is a direct assay based on the principle of radioligand assays.
During an overnight incubation period the I25-I-Insulin in the tracer binds to the Insulin autoantibodies present in the standards and samples. Upon addition of Protein A (which binds to the Fc moiety of the autoantibodies) any labeled antigen-antibody complex is precipitated. After centrifugation the precipitates are counted for I-125.
The concentration of analyte 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 amounts of IAA, the unknown concentration of IAA in patient samples can be determined.

Materials, tools and equipment required

Test tubes (preferably with conical bottom), test tube rack, precision pipettes with disposable tips (20, 25, 50 and 1000 µL), plastic foil, centrifuge, adsorbent tissue, gamma counter, vortex mixer.
Recommended tools and equipment
Repeating pipettes.

Contents of the kit

Component	50 tests kit	100 tests kit
TRACER, ^{125}I-Inzulin <34 kBq/vial, ready to use, 1.5 mL/vial, orange	**1 vial**	**2 vials**
PROTEIN-A, freeze dried, blue.	**1 vial**	**2 vials**
NEGATIVE CONTROL SERUM, ready to use, 0.5 mL/vial. Conc.: 0.04 U/mL	**1 vial**	**1 vial**
STANDARDS, ready to use, 0.5 mL/vial in human serum. Conc.: 0.4, 2, 10, 50 U/mL.	**4 vials**	**4 vials**
CONTROLS, ready to use, 0.5 mL/vial in human serum.	**2 vials**	**2 vials**
RECONSTITUTION BUFFER, ready to use.	**1 vial 6 mL**	**1 vial 6 mL**
PRECIPITATION BUFFER, ready to use.	**1 vial 55 mL**	**1 vial 105 mL**

All kits contain: Quality certificate and Pack leaflet.
Concentration of controls: see quality certificate enclosed.
All components contain 0.1% NaN_3 as preservative.

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, hemolyzed or turbid specimens.

Preparation of reagents, storage

Preparation:
Protein A: Reconstitute each vial with 2.6 mL reconstitution buffer. Mix the contents of the vials thoroughly immediately prior to use.
Storage:
The reconstituted components and the ready to use components can be stored at 2-8 °C.
At this temperature each reagent is stable until expiry date of the kit. The actual expiry date is given on the package label and in the quality certificate.
CAUTION!
Equilibrate all reagents to room temperature except precipitation buffer. Mix all reagents thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide)

1. Label tubes in duplicate for negative control serum (CN), each standard (S1-S4), control serum (CI, CII), sample (P_x) and for total count (TC).
2. Pipette **20 µL** each of STANDARD, CONTROL and SAMPLE into the properly labelled tubes.
3. Pipette **25 µL** of TRACER into each tube.
4. Vortex mix, cover the tubes with plastic foil and incubate tubes **overnight** (18-24 hours) at room temperature.
5. Pipette **50 µL** of PROTEIN A into each tube, except TC. (Mix the content of the Protein A vial thoroughly immediately prior to use.)
6. Vortex mix and incubate tubes for **1 hour** at 4°C.
7. Pipette **1 mL** of **cold** (4°C) PRECIPITATION BUFFER into each tube.
8. Centrifuge the tubes at 2000 x g for 3**0 minutes** at 4°C.
9. Aspirate the supernatant or carefully decant from all tubes by the inversion of the rack, except TC.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the concentrations as described under *Calculation of results*.

Calculation of results

Calculate the average counts per minute (CPM) for each pair of assay tubes.

Calculate percent binding for each standard, control and sample:

$$B/T\ (\%) = \frac{S1\text{-}S4/\ CN,CI\text{-}II\ /\ P_x\ (cpm)}{T\ (cpm)} \times 100$$

Using semi-logarithmic graph paper plot B/T(%) for each standard versus the corresponding concentration of standards. Determine the IAA 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 smoothed spline fittings can be used, similarly to sandwich-type (IRMA) assays.

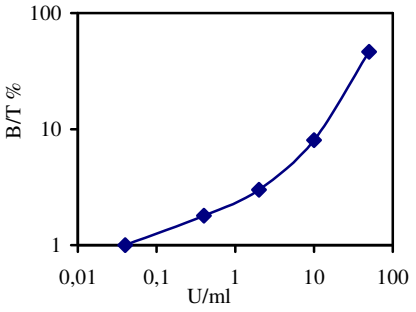
Assay Protocol, Pipetting Guide
(all volumes are in microlitres)

	T	S1-S4	CN,CI-II	P_x
Standards		**20**		
Controls			**20**	
Samples				**20**
Tracer	**25**	**25**	**25**	**25**
Vortex and incubate for **18-24** hours at room temperature				
Protein A		**50**	**50**	**50**
Vortex and incubate for **1** hour at 2-8 °C				
Precipitation buffer (cold)		**1000**	**1000**	**1000**
Centrifuge at 2000 x g for **30** minutes at 4°C				
Aspirate or carefully decant the supernatant and blot the tubes for at least **5** minutes				
Count radioactivity (60 sec/tube)				
Calculate the results				

Typical assay data

Tubes	Mean cpm	B/T%	U/mL
T	29982		
CN	278	0.93	0.04
S1	537	1.79	0.4
S2	897	2.99	2
S3	2416	8.06	10
S4	13951	46.53	50
CI	751	2,5	1.27
CII	2482	8,28	10.34

Typical standard curve



Characterization of assay

Calibration

The units in the RK-50PA IAA kit are arbitrary units.

Sensitivity (lower detection limit)

The analytical sensitivity or minimum detectable limit is calculated by the interpolation of the mean counts of the negative control serum plus 3 standard deviation from the standard curve. Determination was carried out using 15 replicates of the negative control serum response.
The value of analytical sensitivity is 0.12 U/mL.

The functional sensitivity is a measure of the IAA concentration that is significantly different from zero as determined by the inter-assay precision profile (20 % CV).
The value of functional sensitivity is approx. 0.2 U/mL.

Specificity

The high quality of the tracer (125-I iodinated Insulin) does secure in direct assay principle of the test, that only IAA react and that any detectable cross reactions with autoantibodies to IA2, GAD65, Thyroglobulin, thyroidal Peroxidase, to the TSH receptor and Acetylcholine receptor should not exist.

Precision and reproducibility

Intra-assay		Inter-assay	
Mean (U/mL) (n=15)	CV%	Mean (U/mL) (n=10)	CV%
0.8	12	0.75	10
1.5	8.6	1	11
3.5	7.7	2.9	9
8.6	3.5	8.4	7
24.5	1.3	25.3	4

Reference values

IAA	U/mL
negative	< 0.4
positive	≥ 0.4

It is recommended that each laboratory establish its own reference intervals.

Limitations

This assay is not intended to be used in patients receiving insuline treatment because Insuline antibodies may be present in the blood of these patients and may cause false positive results.

Negative test results do not rule out autoimmune diabetes. Autoantibody response varies in individuals.
Presence of a single autoantibody in the absence of clinical symptoms has low predictive value.
Not all individuals with autoantibodies will develop T1DM.

Additional information

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

Precaution

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 both Human Immunodeficiency Virus antibody (Anti-HIV-1, 2), Hepatitis-C antibody (anti-HCV), Hepatitis B surface Antigen (HBsAg) and Treponema Antibody.
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*.

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 116.7 mg.

	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	RBU	Reconstitution Buffer
IVD	*In vitro* diagnostic device	PA	Protein A
	Manufacturer	PBU	Precipitation Buffer
	Radioactive material	REF	Catalogue number

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

IZOTOP

INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: 36-1-392-2577, Fax: 36-1-395-9247