Anti-GAD65 [I-125] RIA KIT

Catalogue numbers:
RK-40P50, kit for 50 determinations
RK-40P100, kit for 100 determinations

Description

The anti-GAD65 [I-125] RIA system provides a direct quantitative determination of autoantibodies to GAD65 in human serum in the range of 0.1-120 U/mL.
Each kit contains material sufficient for measuring one standard curve with controls and 17 patient samples (50 tests kit) or 42 patient samples (100 tests kit) 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).
GAD65 autoantibodies are present in 70-80% of newly diagnosed patients with type 1 diabetes and also in a subset of Type 2 diabetes called latent autoimmune diabetes in adults (LADA).
The combination of tests for anti-GAD65, anti-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 the method

The anti-GAD65 [I-125] RIA kit is a direct assay based on radioligand assay principle.
The ^{125}I-GAD65 in the tracer specifically binds to the anti-GAD65 autoantibodies present in the standards and samples during a two hours incubation period.
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 autoantibodies is directly proportional to the radioactivity measured in the test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of anti-GAD65, the unknown concentration of anti-GAD65 in patient samples can be determined.

Materials, tools and equipment required

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

Recommended tools and equipment

Repeating pipettes (50 and 1000 µL).

Contents of the kit

Component	50 tests kit	100 tests kit
TRACER, ^{125}I-GAD65 <50 kBq/vial, freeze-dried, red.	**1 vial**	**2 vials**
PROTEIN-A, freeze dried, blue.	**1 vial**	**2 vials**
STANDARDS, ready to use, 0.25 mL/vial in human serum.	**6 vials**	**6 vials**
CONTROLS, ready to use, 0.25 mL/vial in human serum.	**2 vials**	**2 vials**
RECONSTITUTION BUFFER, ready to use.	**1 vial 6 mL**	**1 vial 12 mL**
PRECIPITATION BUFFER, ready to use.	**1 vial 55 mL**	**1 vial 105 mL**

Concentration of standards: see vial labels and quality certificate enclosed.
Concentration of controls: see quality certificate enclosed.
All components contain 0.1% NaN_3 as preservative.

Preparation of reagents, storage

Tracer and Protein-A:

Reconstitute each vial with 2.6 mL Reconstitution Buffer. Mix the contents of the vials thoroughly immediately prior to use. Reconstituted Tracer and Protein-A are stable for 2 weeks, stored at 2-8°C.

All other components are stable at 2-8 °C until the 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.

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Sera 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.

Assay procedure

(For a quick guide)

1. Label tubes in duplicate for standards (S1-S6), controls (CI, CII), samples (P_x) and total counts (T).
2. Pipette **20 µL** each of STANDARDS, CONTROLS and SAMPLES into the properly labelled tubes.
3. Pipette **50 µL** of TRACER into each tube. Cover and put aside total counts (T) tubes.
4. Vortex mix, cover the tubes with plastic foil and incubate 2 hours at room temperature.
5. Pipette **50 µL** of PROTEIN A into each tube. (Mix the content of the Protein A vial thoroughly immediately prior to use.)
6. Vortex mix and incubate tubes for 1 hour at room temperature.
7. Pipette **1 mL** of **cold (4°C)** PRECIPITATION BUFFER into each tube.
8. Centrifuge the tubes at 2000 x g for 30 minutes at 4°C.
9. Aspirate the supernatant or carefully decant from all tubes by the inversion of the rack. Leave 5 minutes upside down on absorbent tissue.
10. Count each tube for 60 seconds in a gamma counter.
11. Calculate the concentrations as described under *Calculation of results*.

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

	T	S1-S6	CI-II	P_x
Standards		**20**		
Controls			**20**	
Samples				**20**
Tracer	**50**	**50**	**50**	**50**
Vortex, incubate 2 hours at room temperature				
Protein A		**50**	**50**	**50**
Vortex, incubate 1 hour at room temperature				
Precipitation buffer (cold)		**1000**	**1000**	**1000**
Centrifuge at 2000 x g for **30** minutes at 4°C				
Aspirate or carefully decant the supernatant, leave 5 minutes upside down				
Count radioactivity (60 sec/tube)				
Calculate the 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:

$$B/T\ (\%) = \frac{S1\text{-}S6/\ CI\text{-}II\ /\ P_x\ (cpm)}{T\ (cpm)} \times 100$$

Using semi-logarithmic graph paper plot mean cpm values or preferably B/T(%) for each standard (y axis) versus its corresponding concentration (x axis).

Determine the anti-GAD65 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.

Table 2. Typical Assay Data

Tubes	Mean cpm	B/T%	U/mL
T	31556		
S1	839	2.7	0.1
S2	2468	7.8	1
S3	4029	12.8	3
S4	6602	20.9	10
S5	12994	41.2	35
S6	25488	80.8	120
CI	4058	12.9	3.1
CII	8798	27.9	17.2

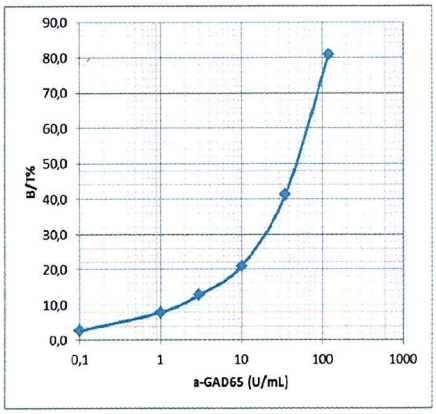

Figure 1.
A typical standard curve
(Do not use to calculate sample values)

Characterization of assay

Calibration

The units in the anti-GAD65 [I-125] RIA kit are arbitrary units.

Detection limit

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 20 replicates of zero standard response.

The value of analytical sensitivity is **0.1 U/ml**.

For the functional sensitivity **0.7 U/mL** was obtained, determined as the value extrapolated to 20 % of the inter-assay imprecision profile obtained from 20 independent runs of serum pools with low anti-GAD65 concentration

Specificity

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

Precision and reproducibility

Four human serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision four human serum pools were measured in duplicates in 20 independent assays.

intra-assay		inter-assay	
Mean U/mL	CV%	Mean U/mL	CV%
0.8	10	0.6	20.7
1.3	9.8	1.2	12.6
8.2	6.8	8.4	5.2
45.9	1.3	45.6	1.1

Expected values

Normal values were determined by measuring 392 presumably healthy blood donors and 398 children samples.

GAD65 autoantibodies	Cut-off U/mL
negative	< 1
grey zone	1 - 2
positive	> 2

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

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.

Limitations

GAD65 autoantibodies may be also present in a rare neurological disorder called Stiff-man syndrome (SMS).

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.

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*.

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 (kit for 50 determinations) or 129 mg (kit for 100 determinations).

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

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