



Instructions for Use


GAD - Antibody Assay

125I-Radioassay for the Quantitative Determination of Antibodies against Glutamic Acid Decarboxylase in Serum



REF	RA102/50	RA103/100
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	50	100
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	2 – 8 °C
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1. Principle of the Test

Insulin-dependent diabetes mellitus (IDDM) is characterised by the presence of several distinct circulating autoantibodies including autoantibodies to insulin and autoantibodies to glutamic acid decarboxylase (GAD). Two isoforms of GAD of molecular weights 65,000 (GAD 65) and 67,000 (GAD 67) have been identified. GAD 65 is the predominant form found in human islets and has been shown to be a major target for autoantibodies in IDDM.

In the assay, test serum samples are incubated, first with ^{125}I -labelled human recombinant GAD 65. This is followed by addition of solid phase protein A to precipitate the labelled GAD - GAD antibody complexes. After centrifugation, the precipitates are counted for ^{125}I and the amount of radioactivity in the precipitates is proportional to the concentration of GAD antibody in the test sample.

2. Precautions

- For in vitro use only.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- This radioactive product assay only be received, stored as used by persons so authorized and by laboratories covered by such authorization. It must not be administered to humans or animals under any circumstances.
- Do not eat, drink or smoke where radioactive materials are being handled.
- Do not pipette by mouth.
- Wear disposable gloves when handling radioactive materials.
- The kit components "Negative and Positive Controls" are made with human serum. All sera used are tested for HIV I/II antibodies and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potentially biohazardous materials.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

4.1 **Tracer** **TRACER** 1(2) vial(s)
125I- labelled GAD 65
2.6 ml, lyophilized, activity < 50 kBq per vial

4.2 **Protein A** **PROTEIN A** 1(2) vial(s)
2.6 ml, lyophilized

4.3 **Assay Buffer** **ASSAY BUFFER** 1 bottle
120 ml, ready for use

4.4 **Standards** **CAL A** – **CAL F** 6 vials
each 150 µl, ready for use
Concentrations:

Standard	A	B	C	D	E	F
U/ml	0	1	3	10	30	300

4.5 **Control 1 & 2** **CONTROL 1** – **CONTROL 2** 2 vials
each 150 µl, ready for use,
contains human serum
Concentrations see QC Certificate

Additional materials and equipment required but not provided:

- Pipettes for 20, 50 µl, and 1 ml
- Plastic tubes and suitable rack
- Centrifuge (preferable refrigerated) capable of at least 3,000 x g
- Suitable device for aspirating or decanting the tubes.
- Vortex mixer
- Gamma counter

5. Sample Collection and Storage

Serum should be used in the assay. Do not use lipemic or grossly haemolized specimen. Repeated freezing and thawing should be avoided. Samples which appear turbid should be centrifuged before assay to remove any particulate material.

Samples can be stored up to one week at 2 - 8 °C or at -20 °C for longer periods.

6. Preparation of Samples and Reagents

6.1 Patient Samples

Allow the patient sera to reach room temperature, mix gently, and centrifuge, if necessary, to remove any particulate material.

6.2 Tracer **TRACER**

Reconstitute the lyophilized tracer with 2.6 ml Assay Buffer (4.3) per vial prior to use and mix carefully.

Store the reconstituted tracer at 2-8 °C for up to 2 weeks.

6.2 Protein A **PROTEIN A**

Reconstitute the lyophilized Protein A with 2.6 ml Assay Buffer (4.3) and mix carefully. Prior to use the suspension has to be mixed thoroughly to ensure a uniform suspension.

Store the reconstituted Protein A at 2-8 °C and use within the shelf life of the kit.

7. Test Procedure

- 7.1 Pipette each 20 μ l standards, controls and patient sera into the corresponding tubes.
- 7.2 Pipette each 50 μ l reconstituted tracer into all tubes.
- 7.3 Mix thoroughly (vortex) and incubate for 2 hours at room temperature (20 - 25 °C).
- 7.4 Mix the Protein A suspension thoroughly immediately prior to use and pipette each 50 μ l Protein A into all tubes (except Totals).
- 7.5 Mix thoroughly (vortex) and incubate for 1 hour at room temperature.
- 7.6 Pipette each 1 ml **cold** (2-8 °C) Assay Buffer (4.3.) into all tubes (except T).
- 7.7 Mix thoroughly and centrifuge for 15 minutes at 3,000 x g, if possible in a refrigerated centrifuge.
- 7.8 Decant or aspirate the supernatant (except T).
- 7.9 Count all tubes for 1 minute in a gamma counter.

8. Calculation of Results

Construct a standard curve by plotting the mean cpm of each Standard versus its corresponding concentration.

The concentration of the Controls and patient samples can then be read off the standard curve.

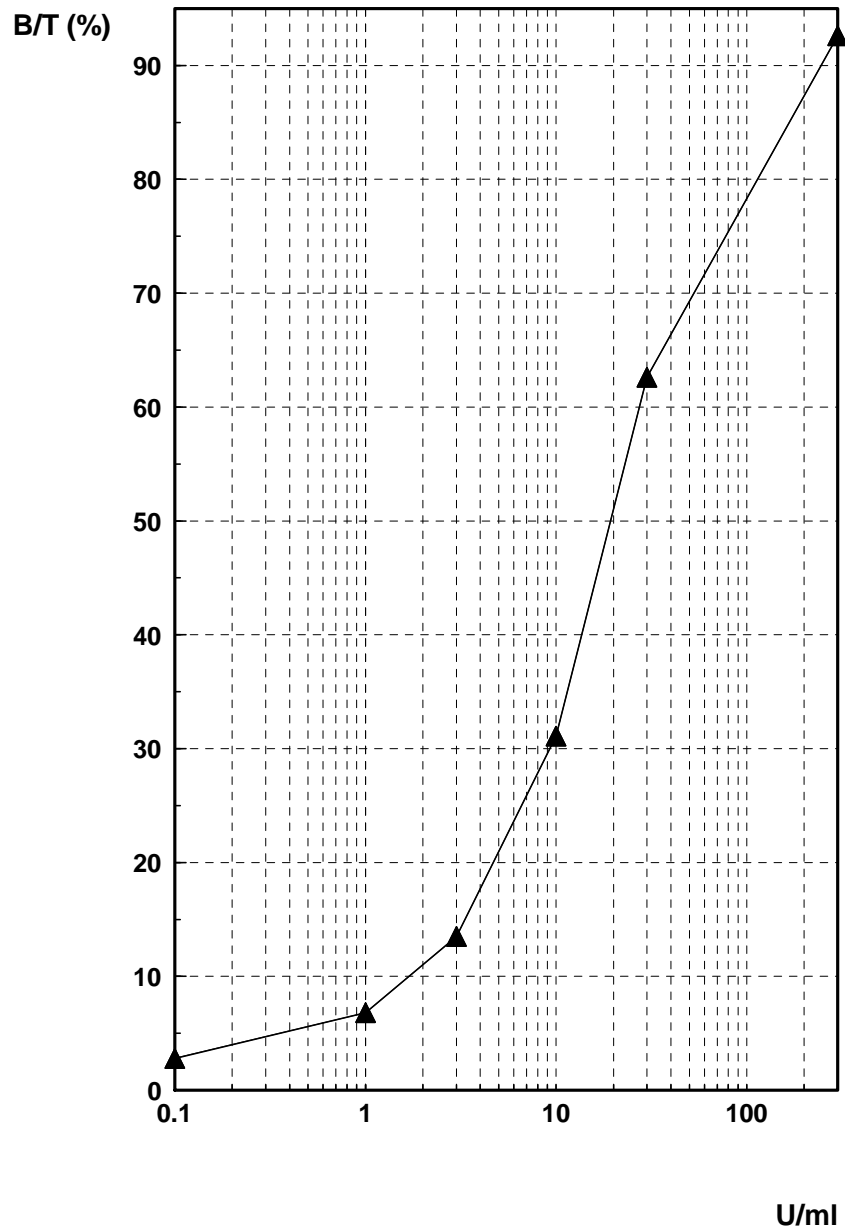
Alternatively, the cpm of the standards, controls, and patient samples can be related to the cpm of the Totals (B/T in %) and used on the y-axis for constructing the standard curve and for reading off the measured concentrations.

9. Typical Example

Typical results are shown in the following table

Sample	cpm1	cpm2	Mean	B/T (%)	U/ml
Total Act.	44,305	44,331	44,318		
Standard A	961	988	975	2.2	0
Standard B	2,685	2,810	2,748	6.2	1
Standard C	6,140	6,003	6,072	13.7	3
Standard D	15,226	14,644	14,935	33.7	10
Standard E	26,893	27,441	27,167	61.3	30
Standard F	41,433	39,758	40,595	91.6	300
Control 1	7,899	8,144	8,022	18.1	4.1
Control 2	25,863	25,369	25,616	57.8	25.5

Typical Standard Curve



10. Reference Ranges

The normal range was determined by measuring the sera of healthy blood donors. From these results a upper normal limit of ≤ 1 U/ml is recommended.

11. Assay Characteristics

Intra-Assay Variation

	Sample 1	Sample 2
N	25	25
Mean (U/ml)	6.4	43
Coefficient of Variation (%)	3.6	3.7

Inter-Assay Variation

	Sample 1	Sample 2
N	25	25
Mean (U/ml)	6.1	43
Coefficient of Variation (%)	4.9	7.0

Clinical Sensitivity and Specificity

Samples from 93 patients diagnosed with type 1 diabetes were assayed. 71% were identified as being positive for GAD antibodies.

100 individual healthy blood donors were assayed. 100% were identified as being negative for GAD antibodies.

In the 2005 DASP study the RIA showed 95% specificity (n=100) and 84% sensitivity (n=50).

Clinical Accuracy

In a study of GAD antibodies in different patient groups, GAD antibodies were not detected in patients with Hashimoto's thyroiditis, myasthenia gravis, or in patients with Addison's disease. 4% (n=27) of patients with Graves' disease were GAD Ab positive

12. Literature

- Schmidli RS; Colman PG; Bonifacio E
Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies. The Second International GADAB Workshop.
Diabetes 44 (1995) 636 - 640
- Petersen JS; Dyrberg T; Karlsen AE; Molvig J; Michelsen B; Nerup J; Mandrup-Poulsen T
Glutamic acid decarboxylase (GAD65) autoantibodies in prediction of beta-cell function and remission in recent-onset IDDM after cyclosporin treatment. The Canadian-European Randomized Control Trial Group.
Diabetes 43 (1994) 1291 - 1296
- E. Hatziagelaki, C. Jaeger, E. Maeser, R.G. Bretzel
GAD 65 antibody but not ICA positivity in adult-onset diabetic patients is associated with early progression to clinical insulin dependency
Acta Diabetol 33 (1996) 291 - 294
- Powell M, Prentice L, Asawa T, Kato R, Sawicka J, Tanaka H, Petersen V, Munkley A, Morgan S, Rees Smith B, Furmaniak J.
Glutamic acid decarboxylase autoantibody assay using 125I labelled recombinant GAD65 produced in yeast
Clin Chimica Acta 256 (1996) 175 - 188

Note:

This kit is manufactured under licence to US patent 5,512,447, European patent 0502 188 B 1 and related patents and patents pending in other countries.

Pipetting Scheme

	T	B0	Standards	Controls	Patients
Standard A μl		20			
Standard B-F μl			20		
Control 1 & 2 μl				20	
Patient Sample μl					20

^{125}I -Tracer μl	50	50	50	50	50
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Mix thoroughly and incubate for 2 hours at room temperature

Protein A μl		50	50	50	50
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Mix thoroughly and incubate for 1 hour at room temperature

Assay Buffer*) ml		1	1	1	1
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*) 2 - 8 °C

Centrifuge (2 - 8 °C) for 15 minutes at min. 3,000 x g

Decant or aspirate the supernatant

Measure for 1 minute in a gamma counter