



Instructions for Use

IA2 - Antibody Assay

125I-Radioassay for the Quantitative Determination of Antibodies against Protein-Tyrosine-Phosphatase (IA2) in Serum



REF	RA101/50	RA104/100
Σ	50	100
$+2$ $^{\circ}\text{C}$	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, autoantibodies to glutamic acid decarboxylase (GAD), and autoantibodies to a protein tyrosine phosphatase, usually referred to as IA2.

In the assay, test serum samples are incubated, first with ^{125}I -labelled human recombinant IA2. This is followed by addition of solid phase protein A to precipitate the labelled IA2-IA2 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 IA2 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 IA2
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 E** 5 vials
each 150 µl, ready for use
Concentrations:

Standard	A	B	C	D	E
U/ml	0	0.75	2	10	50

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) prior to use and mix carefully.

The reconstituted tracer can be stored at 2-8 °C for 2 weeks and must not be frozen.

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.

The reconstituted Protein A can be stored at 2-8 °C and used within shelf life of the kit and must not be frozen.

7. Test Procedure

Allow all reagents excluding assay buffer to stand at room temperature (20 – 25 °C) for at least 30 minutes prior to start of assay.

- 7.1 Pipette each 20 µl standards, controls and patient sera into the corresponding tubes.
- 7.2 Pipette each 50 µl reconstituted tracer into all tubes.
- 7.3 Mix thoroughly (vortex) and incubate for 16 - 20 hours (over night) at 2-8 °C.
- 7.4 Mix the Protein A suspension thoroughly immediately prior to use and pipette each 50 µl Protein A into all tubes (except Totals).
- 7.5 Mix thoroughly (vortex) and incubate for 1hour at 2-8 °C.
- 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.

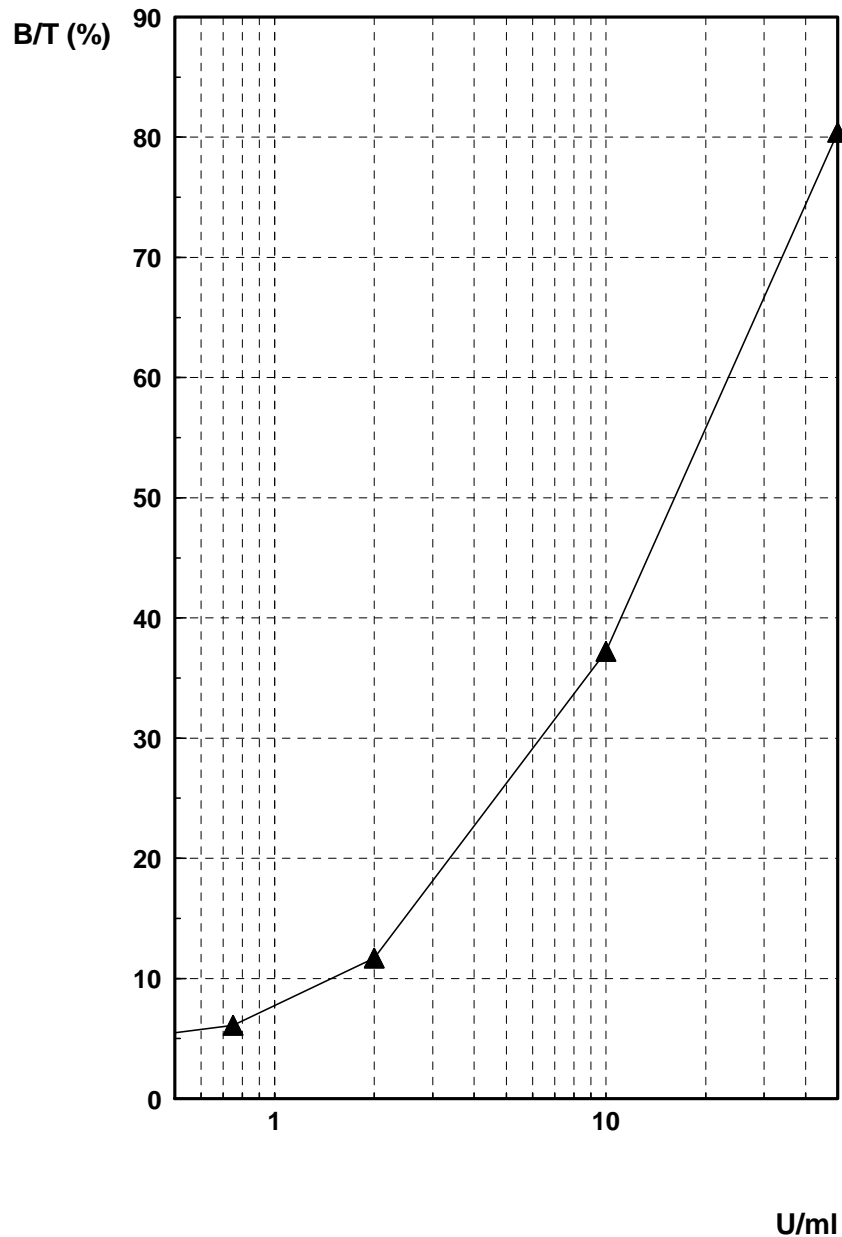
Alternatively, the cpm of the standards, controls, and patient samples can be related to the cpm of the Totals (B/T in %) and used on the y-axis for constructing the standard curve and for reading off the measured concentrations.

9. Typical Example

Typical results are shown in the following table

Sample	cpm1	cpm2	Mean	B/T (%)	U/ml
Total Act.	36,287	36,415	36,351		
Standard A	968	922	945	2.6	0
Standard B	2,251	2,184	2,217	6.1	0.75
Standard C	4,344	4,162	4,253	11.7	2
Standard D	13,677	13,368	13,523	37.2	10
Standard E	29,702	28,750	29,226	80.4	50
Control 1	7,854	8,068	7,961	21.9	4.2
Control 2	23,490	22,894	23,192	63.8	21.4

Typical Standard Curve



10. Reference Ranges

The normal range was determined by measuring the sera of 113 healthy blood donors. From these results a upper normal limit of ≤ 1 U/ml is recommended.

Samples from 217 patients diagnosed with type 1 diabetes were also assayed in the RIA and 47% were identified as being positive for IA2-autoantibodies. In the 2005 DASP study the RIA showed 100% specificity (n=100) and 70% sensitivity (n=50).

11. Assay Characteristics

Intra-Assay Variation

	Sample 1	Sample 2
N	25	25
Mean (U/ml)	6.4	15.1
Coefficient of Variation (%)	2.5	2.8

Inter-Assay Variation

	Sample 1	Sample 2
N	25	25
Mean (U/ml)	6.1	15.0
Coefficient of Variation (%)	3.3	5.3

Sensitivity

The lower limit of detection for the assay is 0.19 U/ml as determined by assaying the lowest standard 20 times, calculating mean and standard deviation and taking 2 standard deviations.

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)

Pipetting Scheme

	T	B0	Standard s	Controls	Patients
Standard A μ l		20			
Standard B-E μ l			20		
Control 1 & 2 μ l				20	
Patient Sample μ l					20

125 I-Tracer μ l	50	50	50	50	50
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Mix thoroughly and incubate for 16 - 20 hours (overnight) at 2 - 8 °C

Protein A μ l		50	50	50	50
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Mix thoroughly and incubate for 1 hour at 2 - 8 °C

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