

Anti-IA2 BL-50-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

Read entire protocol before use. anti-IA2

I. INTENDED USE

Radioligand assay for the determination of autoantibodies to Protein Tyrosine Phosphatase IA2 in serum

II. GENERAL INFORMATION

A. Name: Bio-Line anti-IA2 Kit

B. Catalogue number: BL-50-CT: 50 tests

C. Manufactured by: Bio-Line S.A.

Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

For technical assistance or ordering information contact:

Tel: +32-2-736.62.18.

Fax: +32-2-742.13.15.

III. CLINICAL BACKGROUND

Type 1 diabetes, also known as insulin-dependent diabetes mellitus

(IDDM), results from a chronic autoimmune destruction of the insulin-secreting pancreatic beta cells, probably initiated by exposure of genetically susceptible host to an environmental agent. Autoimmune destruction of beta cells is thought to be completely asymptomatic until 80 -90 % of the cells are lost. This process may take years to complete and may occur at any time.

During the preclinical phase, this autoimmune process is marked by circulating autoantibodies to beta cell antigens. These autoantibodies are present years before the onset of type 1 diabetes and prior to clinical symptoms. Early studies utilized the immunofluorescence test for islet-cell antibodies (ICA), which has been difficult to standardize and is now replaced by a combination of several radioimmunoassays for antibodies against specific beta cell antigens, such as insulin (IAA), glutamic acid decarboxylase (GAD) and tyrosine phosphatase ICA 512 (IA2).

IA2, a member of the protein tyrosine phosphatases family is localized in the dense granules of pancreatic beta cells and the second defined recombinant islet cell antigen. IA2 shares sequence identity with the islet cell antigen 512. The higher frequency of antibodies to IA2 is explained by the presence of autoantibodies directed to the COOH terminus of IA2 which is lacking in the ICA512 molecule. IA2 autoantibodies are present in the majority of individuals with new-onset type 1 diabetes and in individuals in the prediabetic phase of the disease. The appearance of autoantibodies to IA2 seems to be correlated with the rapid progression to overt type 1 diabetes.

The combination of tests for GAD65 and IA2 autoantibodies is highly relevant for risk assessment of type 1 diabetes in children and adolescence. The screening for GAD65 and IA2 autoantibodies detect more than 90 % of subjects at risk for type 1 diabetes and may, therefore, possess the potential to replace ICA technique.

IV. PRINCIPLES OF THE METHOD

anti-IA2 is a direct assay based on the principle of radioligand assays.

Highly purified human recombinant IA2 (intracellular fragment of IA2) is labelled with 125-Iodine. This tracer is used in excess and bound by the IA2 autoantibodies of the sample.

Bio-Line anti-IA2 tracer meets the highest requirements with regard to purity, enzymologic identity, fast reaction kinetics, cross reactivity at zero level and stability. These are the main prerequisites for the specific binding of the tracer and its exclusive recognition by the IA2 autoantibodies of the sample.

By adding Protein A (staphyl. aureus) which binds to the Fc moiety of the autoantibodies, sandwich-type complexes are formed. This solid phase facilitates the simple separation of the bound fraction (B) by centrifugation. After removing the supernatant which contains the nonbound tracer by aspiration or decantation, the radioactivity of the remaining precipitate is measured.

The concentration of IA2 autoantibodies (anti-IA2) in the sample is reflected by the specifically bound tracer amount. The radioactive signal (cpm) of the bound fraction (B) is proportional to the autoantibody concentration.

No immune complex is formed if autoantibodies against IA2 are absent in the sample, as the tracer binds solely to IA2 autoantibodies, but not to Protein A.

A standard curve with a range of 0.1 -50 U/ml is established by measuring cpm's respectively the binding B/T % of the calibrators 1 -5. The anti-IA2 concentration value of the patient's sample is directly read off against this curve.

IX. SPECIMEN COLLECTION AND PREPARATION

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Plasma is also suitable for use in BIO-LINE anti-IA₂.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. For multiple use, initially aliquote samples and keep at - 20 °C.

V. REAGENTS PROVIDED

Tracer (125-I-IA₂, human, recombinant) approx. 50 kBq (1.35 µCi) each per vial	1	vial, lyophilized, reconst.: 2.6 ml buffer B,
Buffer (for reconstitution of components A and C and or washing)	1	vial, 120 ml ready for use
Protein A-Suspension	1	vial, lyophilized, reconst.: 2.6 ml buffer B,
1 - 5 Anti-IA₂-Standards (human serum) <i>Conc.:</i> 0.1; 0.75; 2.0; 10; 50 U/ml)	5	vials, 0.15 ml, each ready for use
I - 2 Anti-IA₂-Control sera (human sera) <i>Conc.:</i> see vial label	2	vials, 0.15 ml, each ready for use

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

BIO-LINE anti-IA₂ has been designed for 50 determinations. This is sufficient for the analysis of 18 unknown samples as well as for calibrators and control sera, assayed in duplicates.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt, all components of the BIO-LINE anti-IA₂ have to be kept at 2 - 8 °C, preferably in the original kit box.

VII. REAGENT PREPARATION

Allow all of the components to reach room temperature prior to use in the assay.

Tracer:

Reconstitute with 2.6 ml buffer B per vial. Reconstituted tracer remains stable for 2 weeks, stored at 2 - 8 °C.

Buffer:

Buffer B is ready for use and serves for the reconstitution of the tracer and the Protein A-suspension as well as for washing.

Protein A-Suspension:

Reconstitute with 2.6 ml buffer B per vial. The reconstituted suspension remains stable for 2 weeks stored at 2 - 8 °C.

Note: *Protein A suspension tends to precipitate in rest, thus agitate bottle end over end gently for 10 - 20 seconds immediately before use. This is not necessary for the short time of taking aliquots for the assay procedure.*

Standards (1-5): Ready for use.

Control sera I-II : Ready for use.

X. PROCEDURE

Use conical tubes for BIO-LINE anti-IA₂ only.

1. Label test tubes* appropriately.
2. Pipette into the corresponding tubes according to assay scheme
 - 20 µl standards,
 - 20 µl control sera,
 - 20 µl patient's samples, each.
3. Add 50 µl tracer (A), each, to **all tubes**, including those for total radioactivity **T**.
Tubes T are now separated until radioactivity is measured.
4. Incubate** over night (at least 18 hours at 4 - 8 °C).
5. Add 50 µl Protein A-suspension (C), each.

(Agitate the suspension gently prior to use - please c.f. section Test Components, preparation before use).
6. Incubate** at least 60 minutes (at room temperature).
7. Add 1 ml buffer (B), each.
8. Centrifuge the tubes for 20 minutes at a minimum of 1500 x g.
9. Aspirate supernatant completely or decant. For removal of any remaining liquid, turn tubes upside down (5 - 10 minutes) and absorb any droplets by tapping on blotting paper.
10. Measure radioactivity of **all tubes including Total activity**.
Recommended counting time: 1 minute

* Use conical tubes.

** Prior to incubation, agitate the tubes briefly in order to ensure homogenous reaction conditions.

XI. CALCULATION OF RESULTS

We recommend log/log processing for best results!

The standard curve is established by plotting the mean cpm-values of the standards 1 – 5 on the ordinate, y-axis, (log. scale) versus their respective anti-IA₂-concentrations on the abscissa, x-axis, (log. scale, as well).

The anti-IA₂ concentrations of the controls and the unknown samples are **directly read off** in U/ml against the respective cpm values.

The respective binding rates B/T (%), related to the total radioactivity T may be used as well for setting up the standard curve.

BIO-LINE anti-IA₂ may be used also with Computer Assisted Analysis using software able to plot log/log curves with spline smoothing, such as for sandwich-type assays (IRMA).

We recommend log/log processing for best results!

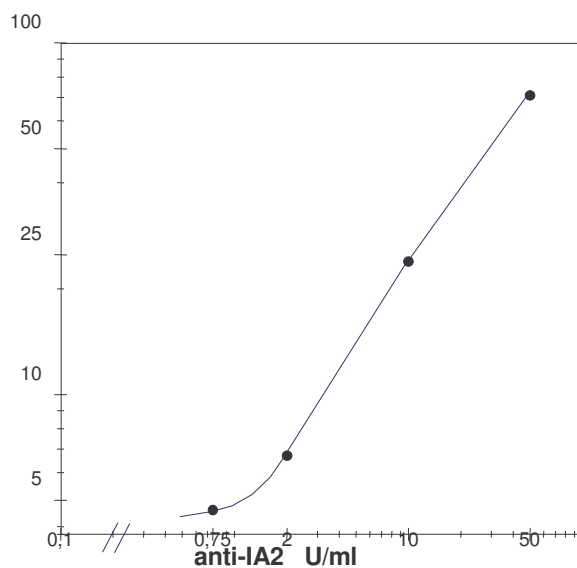
XII. TYPICAL DATA

(approx. 4 weeks before expiry)

Test tubes	cpm (a)	cpm (b)	cpm (mean)	$\frac{B}{T}$ %	U / ml
Total radioactivity T	33533	34136	33833	100 %	---
Standard 1	1078	920	999	3.0	0.10
Standard 2	1616	1532	1574	4.7	0.75
Standard 3	2334	2207	2270	6.7	2.00
Standard 4	8323	7882	8102	23.9	10.00
Standard 5	24341	23612	23976	70.9	50.00
Control I	---	---	---	---	---
Control II	---	---	---	---	---
Patient 1	4330	4322	4326	12.8	4.8

Calculation of patient sample 1: $\frac{B}{T} (\%) = \frac{4326}{33833} \times 100 = 4.8 \%$

Typical standard curve



XIII. PERFORMANCE AND LIMITATIONS

Binding capacity

The maximum binding capacity (B/T %) of the BIO-LINE anti-IA₂ is defined by means of the highest standard (B₅/T %) and is normally found between 65 and 80 %.

$$\frac{B_5}{T} \% = \frac{cpm B_5}{cpm T} \times 100$$

Calibration

At the moment no international standard for IA₂ Ab is available. The units in the BIO-LINE anti-IA₂ are currently arbitrary units. BIO-LINE anti-IA₂ assay based on ¹²⁵I-IA₂ shows good agreement with the ³⁵S labelled IA₂ precipitation assay (r = 0.88; n = 141). BIO-LINE anti-IA₂ passed the 3rd IA₂Ab Proficiency Study of the University of Louisiana, New Orleans, USA, in 1999, with 100 % specificity and 100 % sensitivity.

Parallelism of standards and serum samples

Dilutions of specimen in anti-IA₂ free human serum are determined according to their expected theoretical values with BIO-LINE anti-IA₂.

On the basis of the heterogenous nature of the autoantibody population in human serum and in view of epitope specificity and affinity of the autoantibodies in some cases are not determined the expected theoretical values.

Specificity

The high quality of the tracer (¹²⁵I-IA₂) does secure in direct assay principle of the test, that only anti-IA₂ autoantibodies react and that any detectable cross reactions with autoantibodies to GAD₆₅, Thyroglobulin, thyreoidal Peroxydase, to the TSH receptor and Acetylcholin receptor do not exist.

Sensitivity (lower detection limit)

The most appropriate and statistically reasonable definition of the lower detection limit of any assay is at present the so-called **functional assay sensitivity**.

This functional assay sensitivity generally represents that concentration which corresponds to the 10 % (within-assay) and to the 20 % (between assay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range. Upon correct and thorough performance of BIO-LINE anti-IA₂, this value is found at approx. 0.7 U/ml.

Anti-IA₂ values below this defined level of functional assay sensitivity do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and therefore can not be distinguished from zero due to the statistically necessary certainty.

Anti-IA₂ concentrations above approx. 0.7 U/ml, however, fulfil these criteria and are consequently assessed as valid.

XV. REFERENCE INTERVALS

BIO-LINE anti-IA ₂	
IA ₂ antibodies negative	≤ 0.75 U/ml
IA ₂ antibodies positive	≥ 0.75 U/ml

Normal range was established by evaluation of data from patients with type 1 diabetes and healthy control subjects.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-IA₂ antibodies levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

XVI. PRECAUTIONS AND WARNINGS

Healthy individuals should be tested negative by using the BIO-LINE anti-IA₂.

However, IA₂ autoantibodies may be also present in a rare neurologic disorder, Stiff-man Syndrome (SMS). In sera from patients with SMS IA₂ autoantibodies appear seldom.

Any clinical diagnosis should not be based on the results of in vitro diagnostic method alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Follow the working instructions carefully.

The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

Do not use or mix reagents from different lots.

Do not use reagents from other manufacturers.

Avoid time shift during pipetting of reagents.

All reagents should be kept at 2 - 8 °C before use in the original shipping container.

Some of the reagents contain small amounts (< 0.1 % w/w) of Sodium acid as preservative. They must not be swallowed or allowed to come into contact with skin or mucosae.

Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

We are permitted to transfer the radioactive component of this kit only to laboratories and persons holding a valid licence for handling radioactive material.

Since the kit contains potentially hazardous materials, the following precautions should be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

ASSAY SCHEME

1	Label test tubes*	S 1 - 5	Control sera I - II	Pat.-Sera 1, 2 etc.	T
2	Pipette Standards 1 – 5 Control sera I – II Patient sera	20 µl	20 µl	20 µl	
3	Pipette Tracer (A)	50 µl	50 µl	50 µl	50 µl
4	Incubate**	over night (at least 18 hours) (at 4 - 8 °C)			
5	Pipette Protein A-Suspension (C)	50 µl	50 µl	50 µl	
6	Incubate**	at least 60 minutes (at room temperature)			
7	Pipette Assay Buffer (B)	1 ml	1 ml	1 ml	
8	Centrifuge	20 minutes at 1500 x g			
9	Decant supernatant Or Aspirate supernatant	leave tubes upside down on absorbent paper for 5 to 10 minutes quantitatively			
10	Count radioactivity	Counting time: 1 minute			

* use conical tubes

**Prior to incubation, agitate the tubes briefly in order to ensure homogeneous reaction conditions.