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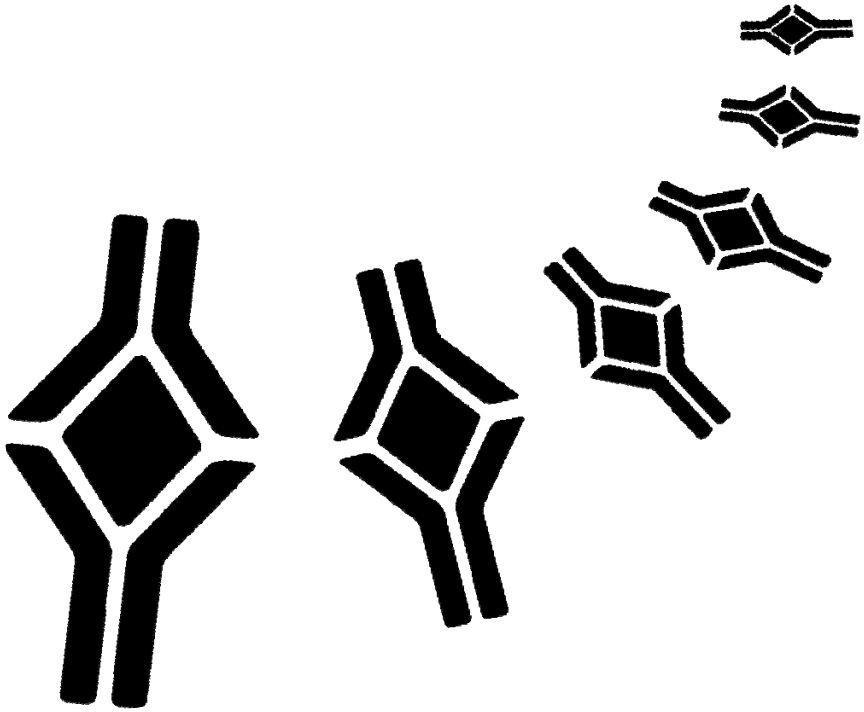


Human 2 Screen Islet Cell Autoantibody ELISA

Product Data Sheet

Cat. No.: R2GI/96R

For Research Use Only

CONTENTS

» This kit is manufactured by: BioVendor – Laboratorní medicína, a.s.

» Use only the current version of Product Data Sheet enclosed with the kit!

1 INTENDED USE

The 2 Screen Islet Cell autoantibody (2 Screen) ELISA kit is intended for use by professional persons only, for quantitative determination of both GAD and IA-2 autoantibodies in human serum. Autoantibodies to pancreatic beta cell antigens are important serological markers of type 1 diabetes mellitus. The antigens recognised by these autoantibodies include insulin, glutamic acid decarboxylase (GAD_{65} kDa isoform) and an islet cell antigen named IA-2 or ICA-512. 2 Screen ELISA allows simultaneous measurement of GAD and IA-2 autoantibodies in the same sample.

2 ASSAY PRINCIPLE

In 2 Screen ELISA, GAD and IA-2 autoantibodies (Ab) in patient sera, calibrators and controls are allowed to interact with GAD_{65} and IA-2 coated onto ELISA plate wells (1^{st} incubation). The samples are then discarded, leaving any GAD or IA-2 autoantibodies in the patient sera, calibrators or controls bound to the GAD_{65} and IA-2 coated wells. A mixture of GAD Biotin and IA-2 Biotin is then added and during a second incubation step (through the ability of GAD and IA-2 autoantibodies to act divalently), a bridge is formed between the GAD_{65} or IA-2 bound to the wells and GAD Biotin or IA-2 Biotin respectively. The amount of GAD/IA-2 Biotin bound is determined in a third incubation step by the addition of Streptavidin Peroxidase, which binds specifically to Biotin. Excess unbound Streptavidin Peroxidase is then washed away and addition of 3,3',5,5' tetramethylbenzidine (TMB) results in formation of a blue colour. This reaction is stopped by addition of stop solution causing the well contents to turn from blue to yellow. The absorbance of the yellow reaction mixture at 450nm is then read using an ELISA plate reader.
A higher absorbance indicates the presence of GAD or IA-2 autoantibody in the test sample. Reading at 405nm allows quantitation of high absorbances.

3 STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. 100µL is sufficient for one assay (duplicate 50µL determinations). Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples. Do not use plasma in the assay. When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at 10-15,000 g in a microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

4 MATERIALS REQUIRED AND NOT SUPPLIED

- Pipettes capable of dispensing 25µL, 50 µL and 100µL.
- Means of measuring out various volumes to reconstitute or dilute reagents supplied.
- Pure water
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450nm and 405nm
- ELISA Plate shaker, capable of 500 shakes/min (not an orbital shaker).

5 PREPARATION OF REAGENTS SUPPLIED

DAY ONE	A	GAD65 and IA-2 Coated Wells 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in foil bag.
		Fit stripwells firmly into frame provided After opening return any unused wells to the original foil packet and seal. Then place foil bag in the self-seal plastic bag with desiccant provided, and store at 2-8°C for up to 16 weeks.
	B	Reaction Enhancer 4 mL Coloured red Ready for use
	C1-6	Calibrators 4, 10, 20, 70, 145 and 450 u/mL (units are NIBSC 97/550) 6 x 0.7 mL Ready for use
	D1	GAD Ab Positive control 0.7 mL Ready for use
	D2	IA-2 Ab Positive Control 0.7 mL Ready for use
	D3	Negative Control 0.7 mL Ready for use

DAY TWO	E	GAD/IA-2 Biotin (GAD Biotin plus IA-2 Biotin) 3 vials lyophilised
		Reconstitute each vial with the amount of GAD/IA-2 Biotin reconstitution buffer (F) shown in the Certificate of Analysis. When more than one vial is used, pool the reconstituted vials and mix gently before use. Use on day of reconstitution.
	F	GAD/IA-2 Biotin reconstitution buffer 2 x 15 mL Coloured blue Ready for use
	G	Streptavidin Peroxidase (SAPOD) 1 x 0.7 mL Concentrated
		Dilute 1 in 20 with diluent for SAPOD (H). For example, 0.5mL (G) + 9.5mL (H). Store at 2 – 8°C for up to 18 weeks after dilution.
	H	Diluent for SAPOD 15 mL Ready for use
	I	Peroxidase Substrate (TMB) 15 mL Ready for use
	J	Concentrated wash solution 125 mL Concentrated
		Dilute 10 X with pure water before use. Store at 2 – 8°C up to kit expiry.
	K	Stop solution 12 mL Ready for use

6 ASSAY PROCEDURE

Allow all reagents to stand at room temperature for at least 30 minutes.

1. Pipette 50 µL of patient sera, calibrators (C1-6) and controls (D1, D2 and D3) into respective wells.
2. Pipette 25 µL of reaction enhancer (B) into each well.
3. Cover the frame and shake the wells for 5 seconds on an ELISA plate shaker (500 shakes per min).
4. Incubate the plate at 2 – 8°C (without shaking) overnight (16-20 hours)
5. After this overnight incubation, aspirate the samples and wash the plate 3 times with wash solution (J) using a plate washer. (If a plate washer is not available, discard the samples by briskly inverting the frame of stripwells over a suitable receptacle, wash the wells 3 times manually and after the final wash invert the frame of wells and tap gently on a clean dry absorbent surface to remove excess wash solution).
6. Pipette 100µL of reconstituted GAD/IA-2 Biotin (E) into each well. Avoid splashing the material out of the wells during addition.
7. Cover the plate, and incubate at 18 - 22 °C for 1 hour on an ELISA plate shaker (500 shakes per min).
8. Repeat wash step 5.
9. Pipette 100µL of diluted streptavidin peroxidase (G) into each well and incubate at room temperature for 20 minutes, on an ELISA plate shaker (500 shakes per min).
10. After the incubation, wash the wells three times with diluted wash solution (J) as in step 5 (in the case of washing manually, use an additional final wash step with pure water to remove any foam).
11. Pipette 100µL of TMB (I) into each well and incubate in the dark at room temperature for 20 minutes without shaking.
12. Pipette 100µL stop solution (K) into each well and shake the plate for approximately 5 seconds on a plate shaker (500 shakes per min). Ensure substrate incubations are the same for each well.
13. Read the absorbance of each well at 405nm and then 450nm using an ELISA plate reader, blanked against a well containing 100µL of TMB substrate (I) and 100µL Stop solution (K) only. This step should be completed within 10 minutes of adding the stop solution (step 12).

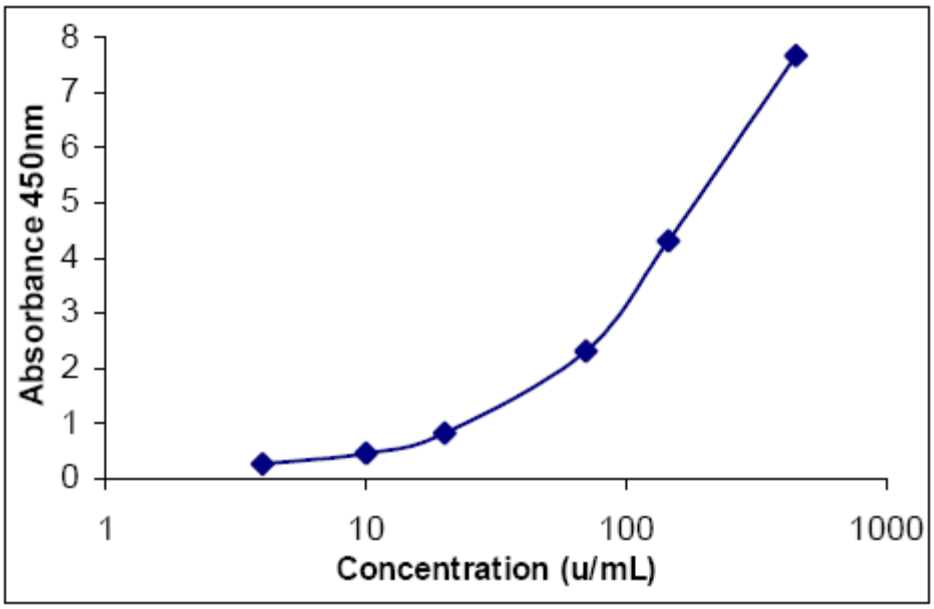
7 RESULTS ANALYSIS

A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the absorbance of the calibrators on the y-axis (linear scale). The GAD and/or IA-2 autoantibody concentrations in patient sera can then be read off the calibration curve. Other data reduction methods can be used. Absorbance readings at 405nm can be converted to 450nm absorbance values by multiplying by the appropriate factor (approximately 3.5, dependant on equipment being used). Values less than 25 u/mL should be read off a 450 nm curve.

8 TYPICAL RESULTS

This data must not be substituted for results obtained in the laboratory.

Calibrator	Absorbance	
u/mL	450nm	405nm
Negative Control	0.120	0.039
4	0.261	0.083
10	0.453	0.133
20	0.818	0.228
70	2.307	0.659
145	4.305	1.230
450	7.662	2.189



Index Calculation

If results are to be expressed as an index, only the 4 u/mL calibrator need be included in the assay (all controls should still be included). The index values are calculated as follows:

$$\text{Index} = \frac{\text{test sample absorbance at 450nm}}{\text{4 u/mL calibrator absorbance at 450nm}}$$

Healthy blood donor sera give index values of less than 1 suggesting that index values of 1 or more can be considered positive for GADAb and/or
IA-2Ab.

9 ASSAY CUT OF

u/mL	
Negative	< 4 u/mL
Positive	≥ 4.0 u/mL

10 CLINICAL EVALUATION

10.1 Clinical Specificity

Sera from 70 healthy blood donors were all negative in the 2 Screen ELISA, although occasional healthy blood donors may have detectable GAD autoantibodies. Autoantibodies to GAD and/or IA2 were detected in 84% (n=216) of samples from patients with type 1 diabetes of various disease durations. In the DASP 2005 study, the 2 Screen ELISA showed 98% (n=100) specificity and 96% (n=50) sensitivity.

10.2 Lower Detection Limit

The kit negative control was assayed 30 times and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 0.17u/mL.

10.3 Inter Assay Precision

Sample	u/mL (n=25)	CV (%)
1	6.6	6.3
2	25.7	4.7

10.4 Intra Assay Precision

Sample	u/mL (n=28)	CV (%)
3	115.2	3.4
4	21.2	4.4

10.5 Clinical Accuracy

There is no detectable cross reactivity with autoantibodies to the TSH receptor, to thyroglobulin, to thyroid peroxidase, to ds-DNA to acetylcholine receptor or rheumatoid factor.

10.6 Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 5mg/mL or intralipid up to 3000 mg/dL.

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for GAD and/or IA-2 autoantibody levels with the assay.

11 SAFETY CONSIDERATION

This kit is intended for *in vitro* use by professional persons only. Follow the instructions carefully. Observe expiry dates stated on the labels and the specified stability for reconstituted reagents. Refer to Materials Safety Data Sheet for more detailed safety information. Material of human origin used in the preparation of the kit has been tested and found non reactive for HIV1 and 2 and HCV antibodies and HBsAg but should, none the less, be handled as potentially infectious. Wash hands thoroughly if contamination has occurred and before leaving the laboratory. Sterilise all potentially contaminated waste, including test specimens before disposal. 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. Some components contain small quantities of sodium azide as preservative. With all kit components, avoid ingestion, inhalation, injection and contact with skin, eyes and clothing. Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

12 ASSAY PLAN

Allow all reagents and samples to reach room temperature before use	
Pipette:	50µL Calibrators, Controls, Patient Sera
Pipette:	25µL Reaction Enhancer
Mix:	Shake for 5 seconds at 500 shakes/min
Incubate	Overnight (16-20) hours at 2 – 8°C (without shaking)
Aspirate/Decant:	Plate
Wash:	Plate three times (dry on absorbent material for manual wash)
Pipette:	100µL GAD/IA-2 Biotin (reconstituted) into each well
Incubate:	1 hour at 18 - 22 °C with shaking at 500 shakes/min
Aspirate/Decant:	Plate
Wash:	Plate three times (dry on absorbent material for manual wash)
Pipette:	100µL SAPOD (diluted 1:20) into each well
Incubate:	20 minutes at room temperature with shaking at 500 shakes/min
Aspirate/Decant:	Plate
Wash:	Plate three times, (additional rinse with pure water and dry on absorbent material for manual wash)
Pipette:	100µL TMB into each well
Incubate:	20 minutes at room temperature in the dark (without shaking)
Pipette:	100µL stop solution into each well and shake for 5 seconds
Read absorbance at 405nm and 450nm within 10 minutes of stop solution addition.	

13 REFERENCES

S. Chen et al
Sensitive non-isotopic assays for autoantibodies to IA2 and to a combination of both IA2 and GAD_{65}.
Clinica Chimica Acta 2005 357: 74-83

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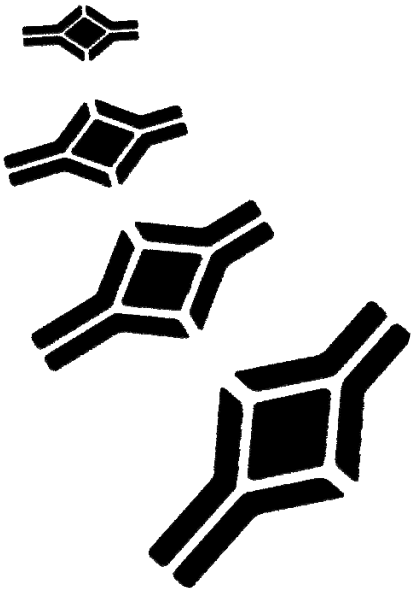

HEADQUARTERS: BioVendor Laboratorní medicína, a.s.	CTPark Modrice Evropska 873	664 42 Modrice CZECH REPUBLIC	Phone: +420-549-124-185 Fax: +420-549-211-460	E-mail:info@biovendor.com Web:www.biovendor.com
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2405 YiYa Tower TianYu Garden, No.150	Lihe Zhong Road Guang Zhou, CHINA	Phone: +86-20-87063029 Fax: +86-20-87063016	E-mail: infoCN@biovendor.com