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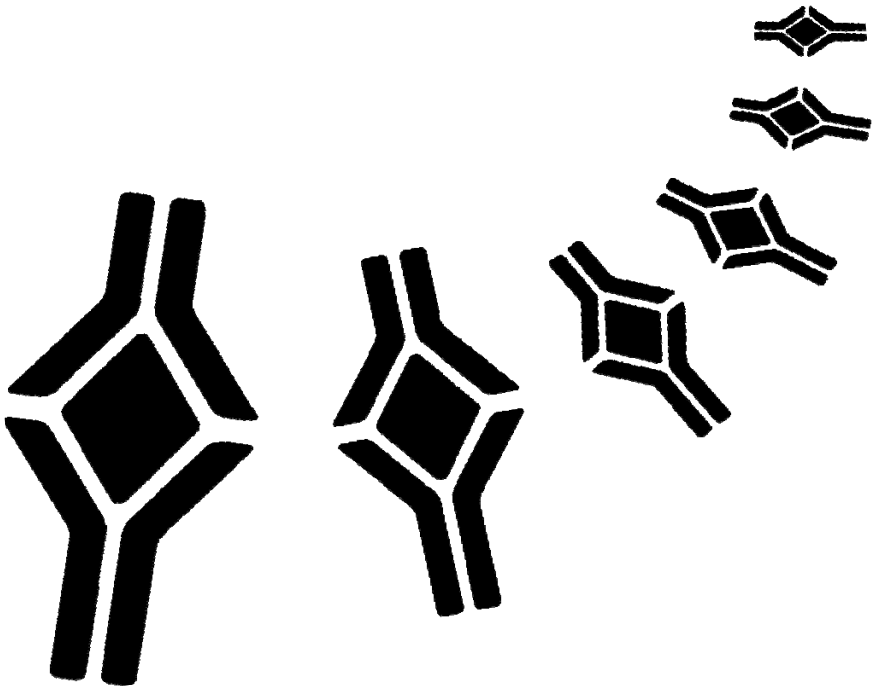


IA-2 Autoantibody ELISA

Product Data Sheet

Cat. No.: RIAE/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 IA-2 autoantibody (IA-2 Ab) ELISA kit is intended for use by professional persons only, for the quantitative determination of IA-2 autoantibodies in human serum.
Autoantibodies to pancreatic beta cell antigens are important serological markers of type 1 diabetes mellitus (type 1 DM). The antigens recognised by these antibodies include insulin, glutamic acid decarboxylase (GAD_{65} kDa isoform) and an islet cell antigen IA-2 or ICA-512.

2 ASSAY PRINCIPLE

In 's IA-2 Ab ELISA, IA-2 autoantibodies in patients' sera, calibrators and controls are allowed to interact with IA-2 coated onto ELISA plate wells. After a 16 - 20 hour incubation, the samples are discarded leaving IA-2 autoantibodies bound to the IA-2 coated wells. IA-2 Biotin is added in a 2nd incubation step where, through the ability of IA-2 autoantibodies to act divalently, a bridge is formed between the IA-2 immobilised on the plate and IA-2 Biotin. The amount of IA-2 Biotin is then 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 405nm and 450nm is then read using an ELISA plate reader. A higher absorbance indicates the presence of IA-2 autoantibody in the test sample. Reading at 405nm allows quantitation of high absorbances. Low values (less than 30 units per mL) should be read off the 450nm calibrator curve.

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. EDTA plasma may be used 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 25 µL, 50µL and 100µL.
Means of measuring out various volumes to reconstitute or dilute reagents.
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).

ELISA Plate cover

5 PREPARATION OF REAGENTS SUPPLIED

A 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 with adhesive tape. Place foil bag in the self-seal plastic bag with desiccant provided, and store at 2-8°C for up to 16 weeks.

B1-6 Calibrators
7.5, 35, 120, 350, 4000 u/mL
(units are NIBSC 97/550)
5 x 0.7 mL
Ready for use

C Positive Control
(see certificate of analysis for concentration range)
0.7 mL
Ready for use

D Negative Control
0.7 mL
Ready for use

E Reaction Enhancer
4.0 mL, coloured red
Ready for use

F IA-2 Biotin
3 vials
Lyophilised
Reconstitute to volume indicated on certificate of analysis using buffer for reconstituting IA-2 Biotin (G). When more than one vial is used, pool the vials and mix gently before use. Store at 2–8°C and use on day of reconstitution.

G Buffer for IA-2 Biotin
2x15 mL, coloured blue
Ready for use

H Streptavidin Peroxidase (SA-POD)
1 x 0.7 mL
Concentrated
Dilute 1 in 20 with diluent for diluting SAPOD (I). For example, 0.5mL (H) + 9.5mL (I). Store at 2–8°C for up to 20 weeks after dilution.

I Diluent for SAPOD
15 mL
Ready for use

J Peroxidase Substrate (TMB)
15 mL
Ready for use

K Concentrated wash solution
125 mL
Concentrated

Dilute 10 X with pure water before use. Store at 2 – 8°C up to kit expiry date.

L Stop solution
12 mL
Ready for use

6 ASSAY PROCEDURE

Allow all reagents to stand at room temperature for
at least 30 minutes before use. A repeating Eppendorf type pipette is recommended for steps 2, 5,8,10 & 11.

1. Pipette 50 µL of patient sera, calibrators (B1-5) and controls (C and D) into respective wells, in duplicate.
2. Pipette 25 µL of reaction enhancer into each well.
3. Cover the frame and shake the wells for 5 seconds at 500 shakes per min then incubate overnight, without shaking, for 16-20 hours at 2–8°C.
4. After incubation, aspirate samples by use of a plate washing machine or discard the samples by briskly inverting the frame of stripwells over a suitable receptacle. Wash the wells three times with diluted wash solution (K), and aspirate the wash by use of a plate washing machine or discard the wash by briskly inverting the frame of stripwells over a suitable receptacle. Tap the inverted wells gently on a clean dry absorbent surface to remove excess wash solution (not necessary when an automatic plate washer is used).
5. Pipette 100 µL of reconstituted IA-2 Biotin into each well. Avoid splashing the material out of the wells during addition.
6. Cover the plate, and incubate at 2-8°C for 1 hour without shaking.
7. Repeat wash step 4.
8. Pipette 100µL of diluted Streptavidin Peroxidase (H) into each well and incubate at room temperature for 20 minutes on an ELISA plate shaker (500 shakes per min).
9. After incubation, discard the samples by briskly inverting the frame of stripwells over a suitable receptacle. Wash the wells three times with diluted wash solution (K) followed by once with pure water (to remove any foam) and tap the inverted wells gently on a clean dry absorbent surface to remove excess wash solution (if a plate washing machine is used, the plate can be washed 3 times with diluted wash solution (K) only).
10. Pipette 100µL of TMB (J) into each well and incubate in the dark at room temperature for 20 minutes without shaking.
11. Pipette 100µL stop solution (L) into each well and shake the plate for approximately 5 seconds on a plate shaker. Ensure substrate incubations are the same for each well.
12. Read the absorbance of each well at 405nm and then 450nm using an ELISA plate reader, blanked against a well containing 100µL of TMB (J) and 100µL Stop solution (L) only.

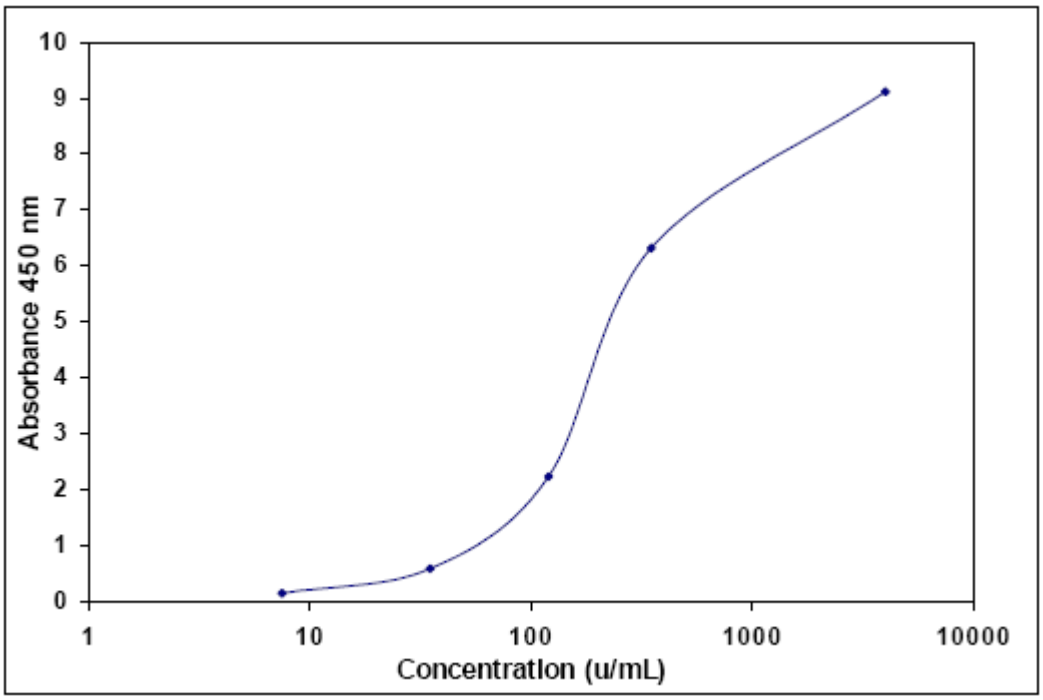
7 RESULTS ANALYSIS

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 IA-2 Autoantibody concentrations in patient sera can then be read off the calibration curve. Other data reduction systems can be used.
Samples with high IA-2 Ab concentrations can be diluted in kit negative control (D). For example, 15 µL of sample plus 135 µL of negative control to give a 10x dilution. Other dilutions (e.g. 100x) can be prepared from a 10x dilution or otherwise as appropriate. Some sera will not divte in a linear way.

8 TYPICAL RESULTS

Example only, not for calculation of actual results.

Calibrator	Absorbance		U/mL
	450nm	405nm	
B1	0.15	0.044	7.5
B2	0.59	0.176	35
B3	2.24	0.666	120
B4	6.32	1.859	350
B5	9.12	2.682	4000
Negative Control D	0.02	0.003	0
Positive Control C	3.05	0.904	158



9 ASSAY CUT OFF

U/mL	
Negative	< 7.5 U/mL
Positive	≥ 7.5 U/mL

10 CLINICAL EVALUATION

10.1 Clinical Specificity and Sensitivity

In the DASP 2005 study the IA-2 Ab ELISA kit showed 99% (n=100) specificity and 66% (n=50) sensitivity.

10.2 Lower Detection Limit

The kit negative control was assayed 20 times, and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 0.15 u/mL.

10.3 Inter Assay Precision

Sample	U/mL	CV (%)
1	19.9	9.8
2	42.7	4.9

10.4 Intra Assay Precision

Sample	U/mL	CV (%)
1	57.6	1.9
2	72.1	3.5

10.5 Clinical Accuracy

Analysis of sera from patients with autoimmune diseases other than type 1 DM indicated no interference from autoantibodies to thyroglobulin; thyroid peroxidase; dsDNA; TSH receptor or from rheumatoid factor.

10.6 Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 5 mg/mL or bilirubin up to 20 mg/dL. Interference was observed with intralipid at 1000 and 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 IA-2 Ab levels

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. Materials of human origin used in the preparation of the kit have 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. 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 and Patient Sera
Pipette:	25 µL Reaction Enhancer
Mix:	Shake on an ELISA plate shaker at 500 shakes/min for 5 seconds
Incubate:	Overnight (16 – 20 hours) at 2 – 8°C, without shaking
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material
Pipette:	100µL IA-2 Biotin (reconstituted) into each well
Incubate:	1 hour at 2 - 8 °C, without shaking
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material
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 and rinse with pure water and tap dry on absorbent material
Pipette:	100µL TMB into each well
Incubate:	20 minutes at room temperature in the dark
Pipette:	100µL stop solution into each well and shake for 5 seconds
Read absorbance at 405 nm and then 450 nm	
It is not necessary to tap dry the plates after washing when an automatic plate washer is used. Also the pure water wash can be omitted from the final wash step when using an automatic washer.	

13 REFERENCES

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European patent 1448 993 B1, Chinese patent ZL0282274.1, Indian patent 226484 and related patents pending in other counties apply.

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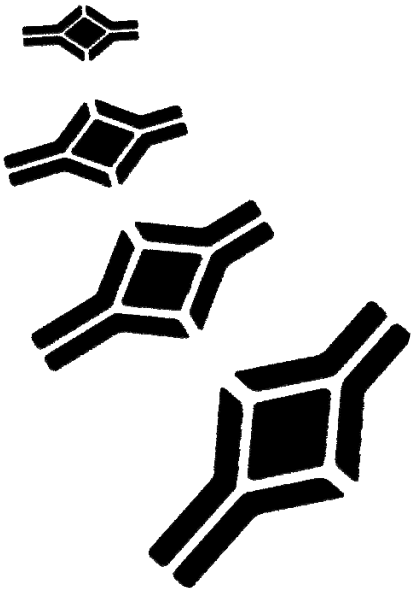

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