



Instructions for Use

IA2 Antibody ELISA

**Enzyme Immuno Assay
for the Quantitative Determination of
Antibodies against IA-2
in Serum**




REF EA114/96



12 x 8













2 – 8
°C

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Symbols

 IVD	In-Vitro-Diagnostic Device		EC Declaration of Conformity
 CONT	Contents		Expiry Date
 LOT	Lot Number		Store
	Manufactured by		Sufficient for
 REF	Catalogue Number		Consult Instructions

Hazard Pictograms



Warning

1. Introduction and Principle of the Test

Insulin-dependent diabetes mellitus (IDDM) is characterised by the presence of several distinct circulating autoantibodies including autoantibodies to insulin, to glutamic acid decarboxylase (GAD₆₅) and an islet cell antigen named IA-2 or ICA 512.

The IA-2 Antibody ELISA depends on the ability of IA-2 autoantibodies to act divalently and form a bridge between IA-2 coated on ELISA plate wells and liquid phase IA-2-biotin. The IA-2-biotin bound is then quantitated by addition of streptavidin peroxidase and a colorogenic substrate (TMB) with reading of final absorbance at 2 wavelengths (450 and 405 nm) to obtain maximum measuring range (7.5 - 4,000 units per ml of WHO reference preparation NIBSC 97/550).

Reference:

S. Chen et al.

Sensitive non isotopic assays for autoantibodies to IA-2 and to a combination of both IA-2 and GAD65 .

Clinica Chimica Acta 2005 357:74-83.

Note:

European patent 1448 993 B1, Chinese patent ZL02822274.1, Indian patent 226484 and Japanese patent 5711449 and US patents US 8,129,132 B2, US 9,435,797 B2 and US 10,488,410 B2 related patents pending in other countries apply.

2. Precautions

- For in vitro use only.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- All reagents of human origin used in this kit are tested for HIV I/II antibodies, HCV 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. Sample Collection and Storage

Serum should be used in the assay. Sera to be analysed should be assayed soon after separation or stored (preferably in aliquots) at 2–8 °C for one week or at -20 °C for longer periods.

About 120 µl is sufficient for one assay. Subsequent freezing and thawing or increase in storage temperature must be avoided. Incorrect storage of serum samples can lead to loss of anti-IA-2 autoantibodies. Do not use grossly haemolysed or lipaemic serum samples. Do not use plasma in the assay.

When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Centrifuge the serum prior to assay (preferably for 5 min at 10-15,000 g in a microfuge) to remove any particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

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.

4. Contents of the Kit

- 4.1 **MT strips** **STRIPS** 12 strips
8 wells per strip
coated with IA-2 antigen,
after opening the pouch, place in the self seal plastic bag
provided, store at 2-8°C and use within 16 weeks


- 4.2 **Calibrator A – E** **CAL A – E** 5 vials
0.7 ml each, ready for use

Concentrations (NIBSC 97/550 units):

Calibrator	A	B	C	D	E
U/ml	7.5	35	120	350	4,000

- 4.3 **Positive Control** **CON +** 1 vial
0.7 ml, ready for use
values for the control are given on the vial label

- 4.4 **Negative Control** **CON -** 1 vial
0.7 ml, ready for use

4.5	IA-2-Biotin freeze-dried; reconstitute as indicated on lot specific qc certificate enclosed in the kit with Reconstitution Buffer (coloured blue) prior to use, store at 2-8°C and use on day of reconstitution	IA-2-BIOTIN	3 vials
4.6	Reconstitution Buffer 15 ml, coloured blue, ready for use for reconstituting IA-2-Biotin	RECONST	2 vial
4.7	Streptavidin-peroxidase (SA-POD) 0.7 ml; 20 x concentrated; dilute 20 x with SA-POD Diluent prior to use; store at 2-8°C and use within 20 weeks	SA-POD	1 vial
			Warning
4.8	SA-POD Diluent 15 ml, ready for use for reconstituting SA-POD	DIL	1 vial
4.9	Reaction Enhancer 4 ml, ready for use (coloured red)	REAC	1 vial
4.10	Substrate 15 ml tetramethyl benzidine (TMB), ready for use	SUB	1 vial
4.11	Wash Buffer 125 ml, 10 x concentrated dilute to 1 litre with pure water before use; store at 2-8 °C after dilution	WASH	1 vial
4.12	Stop Solution 12 ml, ready for use 0.5 M sulphuric acid	STOPP	1 vial

Additional materials and equipment required but not provided:

- Pipettes for 25 µl, 50 µl, 100 µl
- Orbital shaker (up to 500 rpm)
- Pure water
- Microtiter plate reader (405 and 450 nm)

5. Test Procedure

5.1. Preparation of Reagents

MT strips **STRIPS**

Before opening a packet of strip wells, allow it to stand at room temperature for at least 30 minutes. After opening, keep any unused wells in the original foil packet (reseal with adhesive tape) and in the self-seal plastic bag with the desiccant provided. Store at 2-8°C and use within 16 weeks.

IA-2-Biotin **IA-2-BIOTIN**

Reconstitute the contents of one vial to volume indicated on label using Reconstitution Buffer (coloured red) prior to use. 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.

Streptavidin-Peroxidase (SA-POD) **SA-POD**

Dilute 20 x with Streptavidin-Peroxidase (SA-POD) Diluent prior to use. Store at 2-8°C for up to 20 weeks after reconstitution.

Wash Buffer **WASH**

Dilute the contents of one bottle to 1 litre with pure water before use. Store at 2-8°C after dilution until expiry date.

5.2. Assay Procedure

Calculate the number of individual ELISA plate wells needed for the assay. Allow all the reagents supplied, including the appropriate number of packets of strips to reach room temperature (at least 30 min), remove the number of strip wells required and fit them firmly into the frame provided. Negative and Positive Controls in duplicate must always be included in each assay run.

1. Pipette each 50 µl of Calibrator A - E, Negative and Positive Controls and test sera into the appropriate wells (duplicates are recommended).
Pipette 25 µl of IA-2 Reaction Enhancer into each well.
2. Cover the plate and shake for 5 seconds at about 500 rpm. Incubate the plate over night (16 to 20 hours) without shaking at 2-8 °C.

3. Next day reconstitute the IA-2-Biotin, dilute the required amount of Streptavidin-Peroxidase (SA-POD) and dilute the concentrated Wash Buffer.
4. Aspirate or discard the samples from the wells, add 300 µl of Wash Buffer and aspirate or discard again. Repeat two more times. Tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of Wash Buffer.
5. Pipette 100 µl of reconstituted IA-2-Biotin into each well and incubate for 1 hour at **2 – 8 °C** without shaking.
6. After the 1-hour incubation with IA-2 Biotin, aspirate or discard the reagent from the wells, add 300 µl of Wash Buffer and aspirate or discard again. Repeat two more times. Tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of Wash Buffer.
7. Pipette 100 µl of SA-POD into each well and incubate for 20 minutes at room temperature on an ELISA plate shaker (shaking at 500 rpm).
8. Aspirate or discard the reagent from the wells and wash three times with Wash Buffer followed by one wash with pure water to remove any foam from the wells. If a plate washing machine is used, wash 3 times with Wash Buffer only (i.e. omit water wash). Tap the inverted wells gently on a clean dry absorbent surface to remove any droplets.
9. Pipette 100 µl of Substrate (TMB) into each well and incubate for 20 minutes at room temperature in the dark without shaking during which time a blue colour will develop.
10. Stop the substrate reaction by addition of 100 µl of Stop Solution to each well (this will cause the blue colour to turn yellow) and shake the plate for about 5 seconds on a plate shaker to ensure uniformity of the solution in each well.
11. As soon as possible read the absorbance at 450 nm and 405 nm using an ELISA plate reader blanked against a well containing 100 µl Substrate plus 100 µl of Stop Solution.

6. Calculation of Results

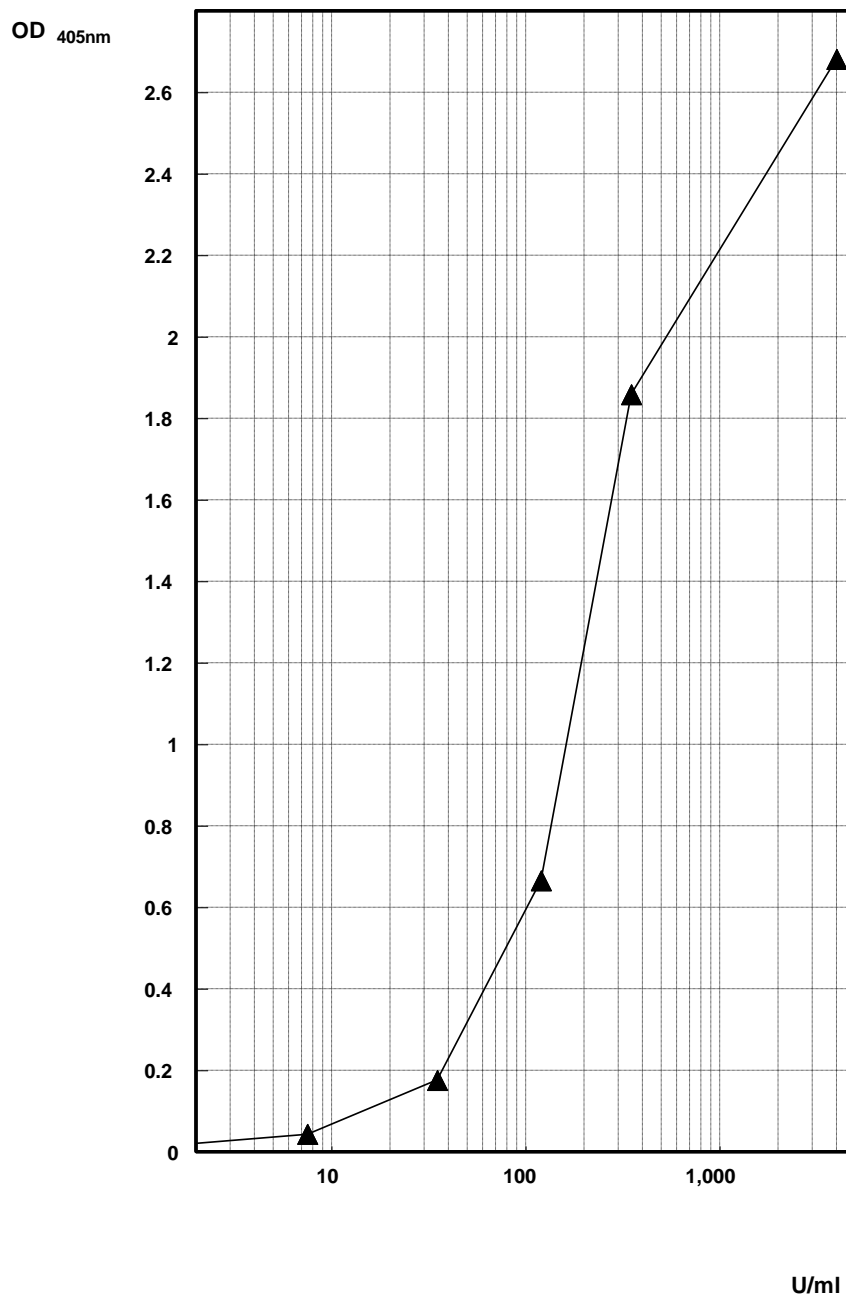
A standard curve can be plotted with absorbance at 450 nm or 405 nm at the vertical axis and \log_{10} Standards concentration on the horizontal axis. Other data reduction procedures can also be used.

Typical Example

In the presence of the negative control, absorbance values at 450 nm should be about 0.1 increasing to above 4 with the higher concentration calibrators. Reading at 405 nm allows quantitation of the high absorbances as shown in the table. Low values should be read off the 450 nm curve.

Typical results are shown in the following table.

Calibrator U/ml	OD _{450 nm}	OD _{405 nm}
Negative Control	0.02	0.003
7.5	0.15	0.044
35	0.59	0.176
120	2.24	0.666
350	6.32	1.859
4,000	9.12	2.682
Positive Control (158 U/ml)	3.05	0.904



7. Expected Values

Healthy blood donor sera give values of less than 7.5 Units per ml (NIBSC 97/550) in the assay suggesting that values of 7.5 U/ml or greater can be considered positive for IA-2 autoantibodies. However, individual laboratories should establish their own cut off values using appropriate control sera.

8. Assay Characteristics

Clinical Sensitivity and Specificity

Results from the DASP 2005 study indicate 99% specificity (n=100) and 66% sensitivity (n=50).

Clinical Accuracy

IA2-Ab were not detected in samples from patients with Hashimoto's thyroiditis, rheumatoid arthritis, Graves' disease or systemic lupus erythematosus.

Precision

The intra and inter assay coefficients of variation (cv) were measured using two samples at different concentration.

intra assay variation		
sample	mean (U/ml)	cv (%)
1	57.6	1.9
2	72.1	3.5

inter assay variation		
sample	mean (U/ml)	cv (%)
1	19.9	9.8
2	42.7	4.9

Pipetting Scheme IA-2 Antibody ELISA

		B ₀	Calibrator	Positive Control	Patients
Calibrator A - E	μl		50		
Positive Control	μl			50	
Negative Control	μl	50			
Patient Sample	μl				50

Reaction Enhancer	μl	25	25	25	25
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Cover, shake 5 sec. and incubate over night (16-20 h) at 2-8 °C

Aspirate / discard and wash three times with each 300 μl Wash Buffer

IA-2-Biotin	μl	100	100	100	100
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Cover and incubate for 1 hour at 2-8 °C without shaking

Aspirate / discard and wash three times with each 300 μl Wash Buffer

SA-POD	μl	100	100	100	100
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Cover and incubate for 20 minutes at RT on a shaker (500 rpm)

Aspirate / discard and wash three times with each 300 μl Wash Buffer
Wash once with 300 μl pure water

TMB-Substrate	μl	100	100	100	100
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Cover and incubate for 20 minutes at RT in the dark without shaking

Stop Solution	μl	100	100	100	100
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5 sec shaking on an ELISA plate shaker

Reading of absorbance at 450 nm and 405 nm