Instructions for Use

IA-2 Autoantibody ELISA

IVD

CE

REF **EIA-4088**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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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), the islet cell antigen IA-2 or ICA-512 and zinc transporter 8 (ZnT8).

2 REFERENCES / LITERATURE

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.

E. Nilson et al. Calcium addition to EDTA plasma eliminates falsely positive results in the GAD Ab ELISA.

Clinica Chimica Acta (2008) 388: 130-134.

K. Rahmati et al. A Comparison of Serum and EDTA Plasma in the Measurement of Glutamic Acid Decarboxylase Autoantibodies (GADA) and Autoantibodies to Islet Antigen-2 (IA-2A), Clin. Lab. 2008 54:227-235.

C. Törn et al. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2.
Diabetologia 2008 51:846-852.

PATENTS

The following patents apply:

US patents US 8,129,132 B2, and US 10,488,410 B2.

3 ASSAY PRINCIPLE

In the 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 hours 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 3rd 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'-tetra-methylbenzidine (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 405 nm and 450 nm is then read using an ELISA plate reader.

A higher absorbance indicates the presence of IA-2 autoantibody in the test sample. Reading at 405 nm allows quantitation of high absorbances (and should be used for concentrations of 120 U/mL or more).

Low values (less than 30 units per mL) should be read off the 450 nm calibrator curve.

The measuring range is 15 – 4000 U/mL.

4 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.

100 µL is sufficient for one assay (duplicate 50 µL 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 about 10,000 rpm i.e. about 10,000 g in a microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

5 MATERIALS REQUIRED AND NOT SUPPLIED

- Pipettes capable of dispensing 25 µL, 50 µL and 100 µL.
- 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 450 nm and 405 nm.
- ELISA Plate shaker, capable of 500 shakes/min (not an orbital shaker).
- ELISA Plate cover

6 PREPARATION OF REAGENTS SUPPLIED

Store unopened kit and components at 2 °C – 8 °C.

A	**IA-2 Coated Wells** 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in foil bag. Ensure stripwells are firmly fitted into frame provided. After opening return any unused wells to the original foil packet with desiccant provided and seal with adhesive tape. Place foil bag in the self-seal plastic bag, and store at 2 °C - 8 °C for up to 16 weeks.
B1-5	**Calibrators** 15, 60, 200, 400, 4000 U/mL, (units are NIBSC 97/550) 5 x 0.7 mL Ready for use
C	**Positive Control** **(see label 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 Immediately before use, reconstitute with the volume indicated on the label using room temperature Buffer for reconstituting IA-2 Biotin (G). When more than one vial is used, pool the vials and mix gently before use.
G	**Buffer for reconstituting IA-2 Biotin** 2 x 15 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, 0.5 mL (H) + 9.5 mL (I). Store at 2 °C - 8 °C for up to 20 weeks after dilution.
I	**Diluent for diluting 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 °C - 8 °C up to expiry date.
L	**Stop solution** 12 mL; Ready for use

7 ASSAY PROCEDURE

Allow all reagents to stand at room temperature (20-25°C) for at least 30 minutes before use, except IA-2 Biotin and IA-2 Biotin reconstitution buffer. Do not reconstitute IA-2 Biotin until step 5 below.

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 is recommended), leaving one well empty for blank.
2. Pipette **25 µL** of reaction enhancer into each well (except blank).
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 °C - 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. Reconstitute IA-2 Biotin (F) with room temperature buffer (G) and pipette **100 µL** into each well (except blank). Avoid splashing the material out of the wells during addition.
6. Cover the plate, and incubate at **18 °C - 22 °C** for 1 hour on an ELISA plate shaker (500 shakes per min).
7. Repeat wash step 4.
8. Pipette **100 µL** of diluted Streptavidin Peroxidase (H) into each well (except blank) 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 **100 µL** of TMB Substrate (J) into each well (including blank) and incubate in the dark at room temperature for 20 minutes without shaking.
11. Pipette **100 µL** stop solution (L) into each well (including blank) and shake the plate for approximately 5 seconds on a plate shaker. Ensure TMB incubations are the same for each well.
12. Read the absorbance of each well at 405 nm and then 450 nm using an ELISA plate reader, blanked against a well containing **100 µL** of TMB (J) and **100 µL** Stop Solution (L) **only**.

8 RESULT 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 [plotted at DRG as a spline log/lin curve (smoothing factor = 0)]. Other data reduction systems can be used. The negative control can be assigned a value of 1.5 u/mL to assist in computer processing of assay results.

Many test sera will have values below 400 u/mL and the 4000 u/mL calibrator need not always be included.

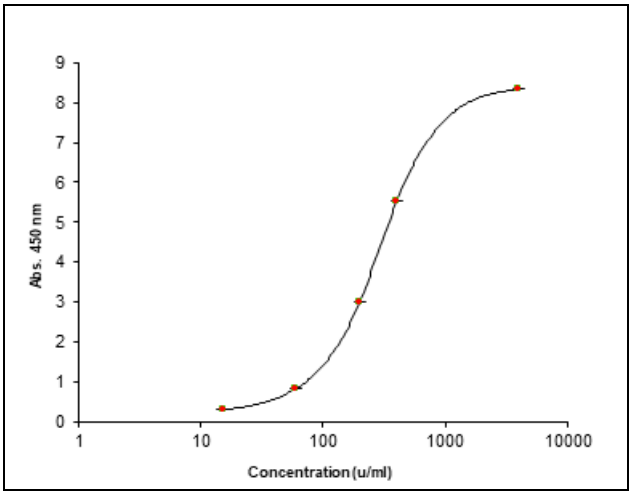
Samples with high IA-2 Ab concentrations can be diluted in kit negative control (D). For example, 15 µL of sample plus 135 µL of negative control to give a 10x dilution.

Other dilutions (e.g. 100x) can be prepared from a 10x dilution or otherwise as appropriate. Some sera will not dilute in a linear way.

TYPICAL RESULTS

(Example only; not for calculation of actual results)

Calibrator	Absorbance		U/mL
	450 nm	405 nm	
B1	0.29	0.085	15
B2	0.82	0.24	60
B3	3.00	0.89	200
B4	5.52	1.62	400
B5	8.33	2.45	4000
Negative Control D	0.094	0.029	0
Positive Control C	1.654	0.487	116



Absorbance readings at 405 nm can be converted to 450 nm absorbance values by multiplying by the appropriate factor (3.4 in the case of equipment used at DRG).

ASSAY CUT OFF

Negative	< 15 U/mL
Positive	≥ 15 U/mL

This cut off has been validated at DRG. However, each laboratory should establish its own normal and pathological reference ranges for IA-2 Ab levels. Also, it is recommended that each laboratory include its own panel of control samples in the assay.

9 CLINICAL EVALUATION

9.1 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.

9.2 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 0.3 U/mL.

9.3 Inter Assay Precision

Sample	U/mL (n=20)	CV (%)
1	143	3.5
2	33	6.6

9.4 Intra Assay Precision

Sample	U/mL (n=25)	CV (%)
1	142	3.6
2	36	5.1

9.5 Clinical Accuracy

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

9.6 Interference

No interference was observed when samples were spiked with the following materials; bilirubin up to 20 mg/dL and Biotin up to 14 µg/mL. Interference was observed with haemoglobin and Intralipid.

10 SAFETY CONSIDERATIONS

Streptavidin Peroxidase (SA-POD) and Reaction Enhancer

Signal word: Warning

Hazard statement(s)

H317: May cause an allergic skin reaction

Precautionary statement(s)

P261: Avoid breathing mist, vapours
P272: Contaminated work clothing should not be allowed out of the workplace
P280: Wear protective gloves/protective clothing/ eye protection/face protection
P302 + P352: IF ON SKIN: Wash with plenty of soap and water
P333 + P313: If skin irritation or rash occurs: Get medical advice/attention
P362 + P364: Take off contaminated clothing and wash it before reuse
P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation

Peroxidase Substrate (TMB)

Signal word: Danger

Hazard statement(s)

H360D: May damage the unborn child

Precautionary statement(s)

P202: Do not handle until all safety precautions have been read and understood

P280: Wear protective gloves/protective clothing/ eye protection/face protection

P308 + P313: IF exposed or concerned: Get medical advice/attention

P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation

Diluent for SA-POD

Hazard statement(s)

EUH208: Contains 2-Chloroacetamide. May produce an allergic reaction.

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 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.
Materials of animal origin used in the preparation of the kit have 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.

11 ASSAY PLAN

Allow all reagents and samples to reach room temperature (20 °C - 25 °C) before use	
Pipette:	**50 µL** Calibrators, Controls and Patient Sera (except blank)
Pipette:	**25 µL** Reaction Enhancer (except blank)
Mix:	Shake on an ELISA plate shaker at 500 shakes/min for 5 seconds
Incubate:	Overnight (16 - 20 hours) at 2 °C - 8 °C, without shaking
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material
Pipette:	**100 µL** IA-2 Biotin (reconstituted with room temperature buffer) into each well (except blank)
Incubate:	1 hour at **18 °C - 22 °C** with shaking at 500 shakes/min (incubation temperature **must not** exceed 22 °C)
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material
Pipette:	**100 µL** SAPOD (diluted 1:20) into each well (except blank)
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:	**100 µL** TMB into each well (including blank)
Incubate:	20 minutes at room temperature **in the dark**
Pipette:	**100 µL** stop solution into each well (including blank) and shake for 5 seconds
Read absorbance at 405 nm and then 450 nm within 5 minutes of stop solution addition.	
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.	

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français	Português
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes	Conformidade Europeia
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation	Consultar as instruções de uso
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro	Dispositivo médico para diagnóstico in vitro
REF	Catalogue number *	Katalognummer *	No. di Cat.	No de catálogo	Référence	Número de catálogo
LOT	Batch code *	Chargen-bezeichnung *	Lotto no	Número de lote	No. de lot	Código do lote
Σ	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests	Suficiente para <n> determinações
	Temperature limit *	Temperaturgrenzwerte *	Temperatura di conservazione	Temperatura de conservacion	Temperature de conservation	Limites de temperatura
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation	Prazo de validade
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant	Fabricante
	Distributor *	Vertriebspartner *	Distributore	Distribuidor	Distributeur	Distribuidor
	Date of manufacture *	Herstellungsdatum *	Data di produzione	Fecha de fabricación	Date de production	Data de fabricação
	Biological risks *	Biologische Risiken *	Rischi biologici	Riesgos biológicos	Risques biologiques	Riscos biológicos
	Caution *	Achtung *	Attenzione	Precaución	Attention	Cuidado
UDI	Unique device Identifier *	eindeutige Produktidentifizierung *	Identificativo unico del dispositivo*	Identificación exclusiva del dispositivo *	Identifiant de dispositif unique*	Identificador único do dispositivo *
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches	
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement	Conteúdo
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité	Volume / Quantidade