Instructions for Use

ICA 2-Screen ELISA

IVD

CE

REF **EIA-4253**

Σ **96**

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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.

Table of Contents

1 INTENDED USE 2
2 REFERENCES / LITERATURE 2
3 ASSAY PRINCIPLE 2
4 STORAGE AND PREPARATION OF TEST SERUM SAMPLES 2
5 MATERIALS REQUIRED AND NOT SUPPLIED 2
6 PREPARATION OF REAGENTS SUPPLIED 3
7 ASSAY PROCEDURE 4
8 RESULT ANALYSIS 4
9 TYPICAL RESULTS 5
10 ASSAY CUT OFF 5
11 CLINICAL EVALUATION 5
12 SAFETY CONSIDERATIONS 6
13 ASSAY PLAN 7

SYMBOLS USED 8

1 INTENDED USE

The ICA 2-Screen Islet Cell autoantibody (ICA 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 antibodies include insulin, glutamic acid decarboxylase (GAD_{65} kDa isoform), the islet cell antigen named IA-2 or ICA-512 and zinc transporter 8 (ZnT8). This ICA 2-Screen ELISA allows simultaneous measurement of GAD and IA-2 autoantibodies in the same sample.

2 REFERENCES / LITERATURE

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

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 patent US 8,129,132 B2 .

3 ASSAY PRINCIPLE

In this ICA 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 (1st 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_{65}-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_{65}-Biotin or IA-2-Biotin respectively. The amount of GAD_{65}/IA-2-Biotin bound is determined in a third incubation step by the addition of Streptavidin Peroxidase (SA-POD), which binds specifically to Biotin.

Excess unbound SA-POD 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 450 nm is then read using an ELISA plate reader. A higher absorbance indicates the presence of GAD or IA-2 Ab in the test sample. Reading at 405 nm allows quantitation of high absorbances.

4 STORAGE AND PREPARATION OF TEST 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 10-15,000 rpm 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 supplied.
- 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	**GAD_{65} and IA-2 Coated Wells** 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in foil bag. Allow to stand at room temperature (20 °C - 25 °C) for at least 30 minutes before opening.
	Ensure strip wells 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 8 months.
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
E	**GAD_{65}/IA-2-Biotin (GAD_{65} Biotin plus IA-2 Biotin)** 3 vials lyophilised
	Reconstitute each vial with the amount of reconstitution buffer for GAD_{65}/IA-2-Biotin (F) shown on the vial label. When more than one vial is used, pool the reconstituted vials and mix gently before use. Use on day of reconstitution.
F	**Reconstitution Buffer for GAD_{65}/IA-2-Biotin** 2 x 15 mL coloured blue Ready for use
G	**Streptavidin Peroxidase (SA-POD)** 1 x 0.7 mL Concentrated
	Dilute 1 in 20 with diluent for SA-POD (H). For example, 0.5mL (G) + 9.5mL (H). Store at 2 °C - 8 °C for up to 18 weeks after dilution.
H	**Diluent for SA-POD** 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 °C - 8 °C up to kit expiry.
K	**Stop Solution** 12 mL Ready for use

7 ASSAY PROCEDURE

Allow all reagents to stand at room temperature (20 °C - 25 °C) for at least 30 minutes before use.

A repeating Eppendorf type pipette is recommended for steps 2, 6, 9, 11 and 12.

Day 1	**1.**	Pipette **50 µL** of patient sera, calibrators (C1-6) and controls (D1, D2 and D3) into respective wells (in duplicate is recommended), leaving one well empty for blank (see step 13).
	2.	Pipette **25 µL** of reaction enhancer (B) into each well (except blank).
	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 °C - 8 °C (without shaking) overnight (16-20 hours)
Day 2	**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 **100 µL** of reconstituted GAD_{65}/IA-2-Biotin (E) into each well (except blank). Avoid splashing the material out of the wells during addition.
	7.	Cover the plate, and incubate at 18 °C - 22 °C for 1 hour on an ELISA plate shaker (500 shakes per min).
	8.	Repeat wash step 5.
	9.	Pipette **100 µL** of diluted SA-POD (G) into each well (except blank) 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 **100 µL** of TMB (I) into each well (including blank) and incubate in the dark at room temperature for 20 minutes without shaking.
	12.	Pipette **100 µL** stop solution (K) into each well (including blank) 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.	Within 10 minutes 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 substrate (I) and **100 µL** Stop solution (K) 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 GAD and/or IA-2 Ab 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 methods can be used.

The negative control (D3) has a concentration of 0 U/mL, but can be assigned a value of 0.4 U/mL to facilitate computer processing of data.

Absorbance readings at 405 nm can be converted to 450 nm 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.

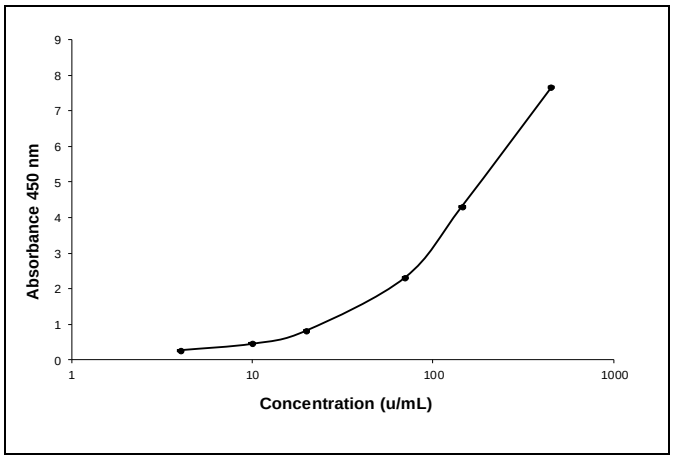
Samples with high GADAb and IA-2Ab concentrations can be diluted in kit negative control (D3).

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.

9 TYPICAL RESULTS

(Example only; not to be used for calculation of actual results)

Calibrator	Absorbance	
U/mL	**450 nm**	**405 nm**
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-2 Ab.

10 ASSAY CUT OFF

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

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

11 CLINICAL EVALUATION

11.1 Clinical Specificity and sensitivity

Sera from 70 healthy blood donors were all negative in the ICA 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 ICA 2-Screen ELISA (EIA-4253) showed 98% (n=100) specificity and 96% (n=50) sensitivity.

11.2 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 0.43 U/mL.

11.3 Intra Assay Precision

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

11.4 Inter Assay Precision

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

11.5 Clinical Accuracy

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

11.6 Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 5 mg/mL, bilirubin up to 20 mg/dL or intralipid up to 3000 mg/dL.

12 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 shelf life for coated wells, reconstituted reagents and diluted reagents.

Refer to 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.

13 ASSAY PLAN

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

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