Product information

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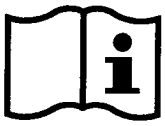

Useŕs Manual

Anti-GAD ELISA

CE

IVD

REF **DEGDE96**

Σ **96 Wells**

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JS
Updated 140304

INTENDED USE

The GAD_{65} autoantibody (GAD Ab) ELISA kit is intended for use by professional persons only, for the quantitative determination of GAD Ab 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 (GAD65 kDa isoform), the islet cell antigen IA-2 or ICA-512 and zinc transporter 8 (ZnT8).

REFERENCES

1. H. Brooking et al A Sensitive non-isotopic assay for GAD_{65} autoantibodies Clinica Chimica Acta 2003 331:55-59
2. 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
3. E. Nilson et al Calcium addition to EDTA plasma eliminates falsely positive results in the RSR GADAb ELISA. Clinica Chimica Acta 388 (2008) 130-134
4. 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) Using the RSR Radioimmunoassay (RIA) and Enzyme Linked Immunosorbent Assay (ELISA) Kits. Clin. Lab. 2008 54:227-235
5. 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

ASSAY PRINCIPLE

In GADAb ELISA, GAD Ab in patients' sera, calibrators and controls are allowed to interact with GAD_{65} coated onto ELISA plate wells. After an 1 hour incubation, the samples are discarded leaving GAD Ab bound to the immobilised GAD_{65} on the plate. GAD_{65}-Biotin is added in a 2^{nd} incubation step where, through the ability of GAD Ab in the samples to act divalently, a bridge is formed between GAD_{65} immobilised on the plate and GAD_{65}-Biotin. The amount of GAD_{65}-Biotin bound is then determined in a 3^{rd} incubation step by 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 yellow. The absorbance of the yellow reaction mixture at 450 nm and 405 nm is then read using an ELISA plate reader. A higher absorbance indicates the presence of GAD Ab the test sample. Reading at 405 nm allows quantitation of high absorbances (and should be used for concentrations of 200 U/mL or more). Low values (less than 10 U/mL) should be read off the 450 nm calibration curve. If it is possible to read at only one wavelength 405 nm may be used. The measuring interval is 5 – 2000 U/mL (units are NIBSC 97/550).

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. 50 µL is sufficient for one assay (duplicate 25 µL determinations). Repeated freeze thawing or increases in storage temperature should 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.

MATERIALS REQUIRED AND NOT SUPPLIED

- Pipettes capable of dispensing 25 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

PREPARATION OF REAGENTS SUPPLIED

Store unopened kit and components at 2-8°C

A. GAD_{65} coated wells
   12 breakapart strips of 8 wells (96 in total) in a frame and sealed in a foil bag. Allow to stand at room temperature (20-25°C) for at least 30 minutes before opening. 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 2-8 °C for up to 16 weeks.

B. Calibrators
   5, 18, 35, 120, 250, 2000 U/mL (units are NIBSC 97/550), 6 x 0.7 mL, Ready for use

C. Positive Control
   (see QC data sheet for concentration range), 0.7 mL, Ready for use

D. Negative Control
   0.7 mL, Ready for use

E. GAD_{65}-Biotin
   3 vials, Lyophilised. Reconstitute each vial with 5.5 mL GAD Biotin reconstitution buffer. When more than one vial is used, pool the vials and mix gently before use. Store at 2-8°C for up to 3 days after reconstitution.

F. Reconstitution Buffer for GAD_{65}-Biotin
   2 x 15 mL, coloured red. Ready for use

G. Streptavidin peroxidase (SA-POD)
   1 x 0.7 mL, Concentrated. Dilute 1 in 20 with diluent for diluting SA-POD. For example, 0.5 mL + 9.5 mL. Store at 2-8°C for up to 16 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 2-8°C up to kit expiry date.

K. Stop solution
   12 mL, Ready for use

ASSAY PROCEDURE

Allow all reagents to stand at room temperature (20-25°C) for at least 30 minutes before use. A repeating Eppendorf type pipette is recommended for steps 4, 7, 10 and 11.

1.	Pipette 25 µL of patient sera, calibrators (B1-6) and controls (C and D) into respective wells, in duplicate, leaving one well empty for blank (see step 12).
2.	Cover the frame and shake the wells for 1 hour at room temperature on an ELISA plate shaker (500 shakes per min.).
3.	Use an ELISA plate washer to aspirate and wash the wells three times with diluted wash solution (J). If a plate washer is not available, discard the well contents by briskly inverting the frame of wells over a suitable receptacle, wash three times manually and finally tap the inverted wells gently on a clean dry absorbent surface.
4.	Pipette 100 µL of reconstituted GAD_{65}- Biotin (E) into each well (except blank). Avoid splashing the material out of the wells during addition.
5.	Cover the frame, and incubate at room temperature for 1 hour on an ELISA plate shaker (500 shakes per min).
6.	Repeat wash step 3.
7.	Pipette 100 µL of diluted Streptavidin Peroxidase (G) into each well (except blank).
8.	Cover the frame and incubate at room temperature for 20 minutes on an ELISA plate shaker (500 shakes per min).
9.	Repeat wash step 3. If manual washing is being carried out use one additional wash step with pure water (to remove any foam) before finally tapping the inverted wells dry.
10.	Pipette 100 µL of TMB (I) into each well (including blank) and incubate in the dark at room temperature for 20 minutes without shaking.
11.	Pipette 100 µL stop solution (K) to each well (including blank) cover the frame and shake 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 450 nm and 405 nm using an ELISA plate reader, blanked against the well containing 100 µL of TMB (I) and 100 µL stop solution (K) only.

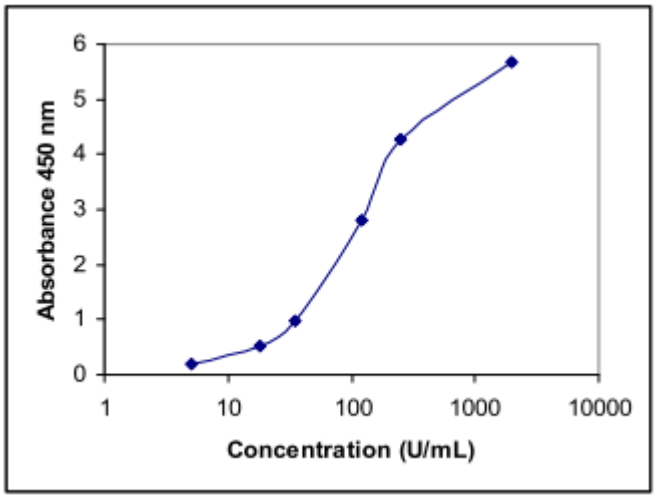
RESULT ANALYSIS

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 Ab concentrations in patients' sera can then be read off the calibration curve [plotted 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 0.5 u/mL to assist in computer processing of assay results. Most test sera will have values below 250 U/mL and the 2000 U/mL calibrator need not always be included. Samples with high Ab concentrations can be diluted in GADAb negative serum or the kit negative control (D) or additional diluent that can be provided. For example, 20 µL of sample plus 180 µL of diluent 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 according to the kit calibrators (standardised against NIBSC 97/550).

TYPICAL RESULTS (Example only, not for calculation of actual results)

Calibrator	A450 nm	Conc. U/ml	A405 nm	Conc. U/ml
B1	0.199	5	0.061	5
B2	0.527	18	0.164	18
B3	0.975	35	0.301	35
B4	2.794	120	0.843	120
B5	4.264	250	1.254	250
B6	5.671	2000	1.668	2000
Negative Control (D)	0.035	0	0.012	0
Positive Control (C)	1.374	49.2	0.418	49.6

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).



ASSAY CUT OFF

Cut off	U/ml
Negative	< 5 U/ml
Positive	≥ 5 U/ml

CLINICAL EVALUATION

Clinical Specificity and Sensitivity

In the DASP 2005 study the GADAb ELISA kit achieved 98 % (n=100) specificity and 92 % (n=50) sensitivity.

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 0.57 U/ml.

Inter Assay Precision

Sample	U/ml (n=20)	CV (%)
A	97	5.7
B	21	5.2
C	5.7	6.4

Intra Assay Precision

Sample	U/ml (n=25)	CV (%)
1	97	7.3
2	20	8.5
3	7.0	3.5

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).

Clinical Accuracy

Analysis of sera from patients with autoimmune diseases other than type 1 DM disease indicated no interference from autoantibodies to thyroglobulin or thyroid peroxidase (n=10) or TSH receptor (n=20). One sample positive for dsDNA (n=10) and one sample positive for rheumatoid factor (n=30) were positive for GAD Ab.

Interference

No interference was observed when samples were spiked with the following materials; haemoglobin at 5 mg/mL, bilirubin at 20 mg/dL 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 Ab levels.

SAFETY CONSIDERATIONS

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. 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. Sterilize 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 should be handled as potentially infectious. Some components contain small quantities of sodium azide as preservative. As with all kit components, avoid ingestion, inhalation, injection or 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.

ASSAY PLAN

Allow all reagents and samples to reach room temperature (20-25°C) before use	
Pipette:	25 µL Calibrators, Controls and Patient Sera (except blank)
Incubate:	1 hour at room temperature on an ELISA plate shaker at 500 shakes/min
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material[1]
Pipette:	100 µL GAD_{65}-Biotin (reconstituted) into each well (except blank)
Incubate:	1 hour at room temperature on an ELISA plate shaker at 500 shakes/min
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material[1]
Pipette:	100 µL SA-POD (diluted 1:20) into each well (except blank)
Incubate:	20 Minutes at room temperature on an ELISA plate shaker at 500 shakes/min
Aspirate/Decant:	Plate
Wash:	Plate three times, rinse with pure water and tap dry on absorbent material[1]
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 450 nm and 405 nm	
[1] It is not necessary to tap dry the plates after washing when an automatic plate washer is used. The pure water wash can be omitted when using an automatic washer.	

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità