ImmunoDiagnostics Limited

Glutamic Acid Decarboxylase (GAD65) Autoantibody ELISA kit

Catalogue Number: 31840

For the quantitative determination of human anti-GAD65 ELISA (IgG class antibodies) in serum

This package insert must be read in its entirety before using this product

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Glutamic Acid Decarboxylase (GAD) Autoantibody ELISA kit from ImmunoDiagnostics

Introduction

Immunodiagnostics (IMD) GAD65 auto-antibody ELISA kit is dedicated to the in vitro determination and quantitative of GAD65 auto-antibody. This product is recommended for clinical and research use only. GAD65 autoantigen is one of the most important serological markers for diagnostics of insulin-dependent diabetes mellitus (IDDM) and latent autoimmune diabetes (LADA). Besides GAD65, autoantibodies to beta-cells also included ICA, IA2 and ZnT8. IDDM is highly prevalent in Europe and North American. Epidemiological study shows that there is a gradual rise of prevalence in China, approximately 10% of Chinese people are suffering from IDDM. Furthermore, GAD65 is also relevant with Stiffman syndrome(SMS).

Assay Principals

In IMD's GAD65 Elisa, recombinant GAD65 is coated onto the ELISA plates and react with GAD65 antibody in human sera and calibrators. After 1 hour incubation, GAD65 antibody specifically binds to the immobilized GAD65. The unbound component in serum were discarded and washed away. Then GAD65-Biotin is added to each well and incubated for 1 hour. In this step, GAD65 antibody works as a connector between immobilized GAD65 and GAD65-Biotin. Then the plates were washed to remove unbound GAD65-Biotin. In 3rd step, streptavidin horseradish peroxidase (STV-HRP) is allowed to specifically binds with biotin. With the additional of substrate 3,3′,5,5′-Tetramethylbenzidine (TMB), the amount of GAD65 antibody will be converted into color signal (blue). The higher concentration GAD65Ab presented in sera, the stronger blue color change. The reaction will be stopped by 2M H_2SO_4, the blue color will turn yellow. The absorbance of yellow reaction mixture is measured by plate reader at 450nm and 405nm. The measuring interval is 5-120IU/ml (IU: International units are WHO standard NIBSC 97/550)

Storage and Preparation

All the sera for analysis should be aliquoted and stored at -20C or below after collected. All the samples are recommended to avoid freeze and thaw for more than 3 times. 50µl is sufficient for one test (duplicate of 25ul test). Lipemic or hemolyzed sera are not recommended to applied on this kit. Plasma are also not recommended to applied on this assay. When required, bring the test serum samples to room

temperature and vortex to ensure homogeneity. Then centrifuge the serum samples at 10-15,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if your sera are cloudy and containing particles.

Symbols

	Manufacturer		EC Declaration of Conformity
	Expiry date		Consult Instruction
	Lot number		Store
	Catalog number		Caution
	In Vitro Diagnostic Device		Bio Hazard
	Negative control		Positive control

Materials not supplied

Pipettes capable to dispense 1000µl, 100µl and 25µl.
Multi-channel pipettes
Shaker capable to shake 500rpm/min.
Plate reader capable to measure at 450nm and 405nm
Distil water

Reagent and materials supplied in IMD GAD65 ELISA kit

A	GAD65 coated Elisa plate	12 strips with 8 wells (96 wells in total) in a frame and sealed in a foil bag. Ensure all the strips are firmly fitted into the frame provided. After opened, return unused strips to the original foil bag and seal with zip. Store the foil bag at 2-8C for up to 16 weeks.
B 1-5	Calibrators	1ml x 6 5, 10, 18, 35, 120 (IU/ml) (International units are WHO standard NIBSC 97/550) Ready to use
C	Positive control	1 ml x 1 Ready to use
D	Negative control	1 ml x 1 Ready to use

E	GAD65-biotin	3Vials Lyophilized Reconstituted each vial to 4.5ml with IMD detection buffer. Store at 4C for up to 3day after reconstitution.
F	Detection buffer (reconstitution buffer for GAD65-biotin)	15ml x 1 Pale yellow Ready to use
G	20 x Streptavidin horseradish peroxidase (STV-HRP)	1ml x 1 Dilute with dilution buffer
H	Dilution buffer (for STV-HRP)	15ml x 1 Pale yellow Ready to use
I	3,3′,5,5′-Tetramethylbenzidine (TMB)	Ready to use Equilibrate to room temperature for 15 minute before use
J	Stop solution (2M H_2SO_4)	15ml x 1 2M H_2SO_4 Ready to use
K	10X Wash buffer	50ml x 1 Dilute with distill water
L	Empty eppendorf tube	10 tubes Reconstituted detection can also be aliquoted and stored at -80 or-20 with less than 5 freeze and thaw cycle.
M	Frame cover	4 slides

Assay Procedure

Please pre-balance all the reagent to room temperature (20-25°C) for at least 30 minutes before use.

Step 1	Pipette 25µl calibrators (B1-5), controls (C and D) and sera to GAD65 coated wells (A) respectively
Step 2	Cover the frame with frame cover (M) and shake the wells at room temperature (500 rpm) for 1 hour on a shaker
Step 3	Dilute wash buffer with distill water (1:10)
Step 4	Reconstitute lyophilized GAD65-biotin (E) to 4.5ml with detection buffer (F)
Step 5	Discard samples on absorbent materials, wash the wells with 350 µl 1X wash buffer for 3 times. Finally tap the inverted plate on clean and dry absorbent materials.
Step 6	Pipette 100 µl reconstituted GAD65-biotin to each well.

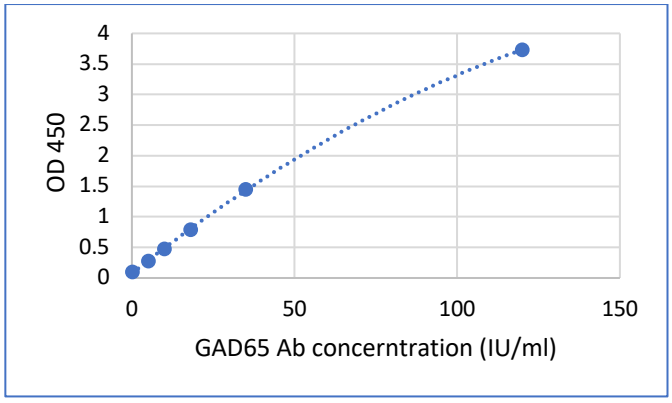
Step 7	Cover the frame and shake the wells at room temperature (500 rpm) for 1 hour on a shaker
Step 8	Dilute STV-HRP (G) with dilution buffer (H) at a ratio of 1:20.
Step 9	Discard detection on absorbent materials, wash the wells with 350 µl 1X wash buffer for 3 times. Finally tap the inverted plate on clean and dry absorbent materials.
Step 10	Pipette 100 µl diluted STV-HRP to each well
Step 11	Cover the frame and shake the wells at room temperature (500 rpm) for 20minute on a shaker
Step 12	Discard samples on absorbent materials, wash ELISA plate with 350 µl 1X wash buffer for 4 times. Finally tap the inverted plate on clean and dry absorbent materials.
Step 13	Pipette 100 µl TMB (I) to each well and then place the ELISA plate in dark at room temperature for 15 minute without shaking.
Step 14	Pipette 100 µl stop (J) solution to each well
Step 15	Read the absorbance of each well at 450nm and 405nm with ELISA plate reader.

Data Analysis

A calibration curve can be established by plotting calibrator concentration on x-axis (log-scale) against the absorbance of the calibrators on the y-axis(linear scale). The GAD65 antibody concentrations in sera can be read off the calibration curve. Negative control can be assigned a value of 0.5IU/ml to assist the statistical software to process the data analysis. Samples with high GAD65 antibody concentration can be diluted with GAD65 antibody free serum or FBS.

Typical Standard Curve

GAD65 Ab concentration (IU/ml)	OD450	OD 405
120	3.732	1.0865
35	1.445	0.439
18	0.794	0.2565
10	0.4785	0.168
5	0.276	0.1125
0	0.096	0.0655



[Assay cut-off value]

<10 IU/ml	Negative
≥10 IU/ml	Positive

This cut-off value has been validate at IMD. However, it is recommended that each laboratory should establish its own normal and pathological reference range for GAD65 antibody level. Furthermore, it is also recommended that each laboratory should include its own panel of control samples in the assay.

[Clinical evaluation]

Clinical Specificity and sensitivity
In the clinical validation at IMD, the IMD GADA ELISA kit achieve 82.5% sensitivity (n=40) and 91.12% (n=80) specificity.

Inter assay precision

Sample	IU/ml (n=6)	CV(%)
A	120	6.75%
B	18	6.66%
C	5	7.26%

Intra assay precision

Sample	IU/ml (n=6)	CV(%)
A	120	6.61%
B	18	4.63%
C	5	9.65%

References:

Stenström, Gunnar, et al. "Latent autoimmune diabetes in adults: definition, prevalence, β-cell function, and treatment." Diabetes 54.suppl 2 (2005): S68-S72.

Schmidli, Robert S., Peter G. Colman, and Ezio Bonifacio. "Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies: the Second International GADAB Workshop." Diabetes 44.6 (1995): 636-640.

Moffett, Ashley, et al. "Variation of maternal KIR and fetal HLA-C genes in reproductive failure: too early for clinical intervention." Reproductive biomedicine online 33.6 (2016): 763-769.