GAD65 ELISA

For the quantitative and qualitative determination of antibodies against human glutamate decarboxylase 65kDa isoform (GAD65) in human serum

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 35-GADHU-E01
Size: 96 wells
Version: RUO: 2018-09-26 (V004) – ALPCO 1.0

1. Intended Use

The GAD65 ELISA GAD65 SL is a solid phase enzyme immunoassay employing recombinant human glutamate decarboxylase 65kDa isoform (GAD65) for the quantitative and qualitative detection of antibodies against human GAD65 in human serum. For Research Use Only. Not for use in Diagnostic Procedures.

2. Principle of the Assay

Serum samples are diluted 1:4 and incubated in the microtiter plates coated with the specific antigen. Antibodies, if present in the sample, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards GAD65 Biotin is incubated. During this step GAD65 autoantibodies form a bridge between the GAD65 immobilized on the plate and the GAD65 Biotin in the liquid phase. Unbound GAD65 is washed off in the following step. The amount of bound GAD65 Biotin is then determined by the addition of streptavidin-peroxidase (conjugate) that binds to biotin. Unbound streptavidin-peroxidase is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is proportional to the initial concentration of the respective antibodies in the sample.

3. Kit Contents

To Be Reconstituted				
Item	**Quantity**	**Cap Color**	**Solution color**	**Description/Contents**
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
GAD65 Biotin	3 vials	White	-	Biotin conjugated to recombinant human GAD65; bovine serum albumin (BSA), lyophilized
Ready to Use				
Item	**Quantity**	**Cap color**	**Solution color**	**Description/Contents**
Reconstitution Buffer	1 x 20ml	Red	Red	PBS, bovine serum albumin (BSA)
Negative Control	1 x 1ml	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1ml	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

Cut-off Calibrator	1 x 1ml	Blue	Yellow	Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1ml	White	Yellow *	Concentration of each calibrator: 0, 25, 75, 125, 250, 500 IU/ml. Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate	1 x 15ml	Blue	Blue	Streptavidin coupled to horseradish peroxidase; bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
Materials Required, but not provided				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), absorbent paper. This test is designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Please note: Reconstituted GAD65 Biotin must be used on day of reconstitution. Once prepared, other reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate must be used within the expiration date indicated on each component. Avoid exposing TMB solution to light. Store microplates in designated foil, with desiccant, and seal tightly.

5. Precautions

5.1 Health hazard data

This product is for research use only. Not for use in diagnostic procedures. Only staff trained and specially advised in the methods of ELISA techniques may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions
This kit contains potentially hazardous components. Though kit reagents are not classified as being irritants to eyes and skin it is recommended to avoid contact with eyes and skin and to wear disposable gloves.

WARNING! Calibrators, controls, and buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or absorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with plenty of water to

prevent azide build-up. Please refer to decontamination procedures as outlined by the CDC or other local/national guidelines.

Do not smoke, eat, or drink when manipulating the kit. Do not pipette by mouth.

All biological source material used in kit reagents has been tested by approved methods and found negative for HBsAg, Hepatitis C, and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Handle kit controls, standards, and samples as if capable of transmitting infectious diseases and according to national requirements.

This kit contains material of animal origin as stated in the materials included, handle according to national requirements.

5.2 General directions for use

- In case that the product information, including the labeling, is defective or incorrect please contact ALPCO.
- Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.
- Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well, and follow the recommended incubation scheme for optimum performance of the assay.
- **Incubation: It is recommended to test performance at 30°C/86°F for automated systems.**
- Never expose components to higher temperatures than 37°C/98.6 °F.
- Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used previously with other reagents.

6. Sample Collection, Handling, and Storage

It is preferable to use freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolyzed, or bacterially contaminated samples. Sera with particles should be cleared by low-speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry, and empty tubes.

After separation, the serum samples should be used during the first 8 hours, respectively stored tightly closed at 2-8°C/35-46°F for up to two days (48 hours), or frozen at -20°C/-4°F for longer periods. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4).

7. Assay Procedure

7.1 Preparations prior to starting

Only prepare reagents which will be used on the same day!
Dilute concentrated reagents:

- Dilute the concentrated sample buffer 1:5 with distilled water (e.g., 20 mL plus 80 mL).
- Dilute the concentrated wash buffer 1:50 with distilled water (e.g., 20 mL plus 980 mL).
- To avoid mistakes, it is suggested to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:4 with 1x working sample buffer e.g. 225 µl sample buffer (1x) + 75 µl serum. Mix well!

Reconstitute and dilute GAD65 Biotin:
Reconstitute lyophilized GAD65 Biotin in two steps: First, add 1 ml reconstitution buffer to one vial, let it stand for 5 minutes and mix it until GAD65 Biotin is completely dissolved. Make sure that no remaining lyophilized or reconstituted GAD65 Biotin in the cap. In the second step, transfer 1 ml reconstituted GAD65 Biotin to 5 ml reconstitution buffer and mix well. One vial of reconstituted GAD65 Biotin is sufficient for six strips. If more strips are used in one test, the two vials of lyophilized GAD65 Biotin must be reconstituted and diluted as described above and then combined. Mix well!

Washing:
Prepare 20 mL of 1x working wash buffer per 8 wells or 200 mL for 96 wells

- e.g., 4 mL concentrate plus 196 mL distilled water.

Automated washing:
Consider excess volumes required for setting up the instrument and the dead volume of robotic pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean absorbent paper. Pipette 300 µL of 1x working wash buffer into each well, wait 20 seconds. Repeat the whole procedure two more times.

Microplates:
Calculate the number of wells required for the test. Remove unused wells from the frame. Replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Suggested Layout

The following layout for pipetting the calibrators, controls, and samples is suggested:

	For quantitative interpretation				For qualitative interpretation			
	1	**2**	**3**	**4…**	**1**	**2**	**3**	**4…**
A	Cal A	Cal E	S1		NC	S2		
B	Cal A	Cal E	S1		NC	S2		
C	Cal B	Cal F	S2		CC	S3		
D	Cal B	Cal F	S2		CC	S3		
E	Cal C	PC	S3		PC	…		
F	Cal C	PC	S3		PC			
G	Cal D	NC	…		S1			
H	Cal D	NC	…		S1			

Cal A: Calibrator A Cal D: Calibrator D PC: Positive Control S1: Sample 1

Cal B: Calibrator B	Cal E: Calibrator E	NC: Negative Control	S2: Sample 2
Cal C: Calibrator C	Cal F: Calibrator F	CC:Cut-Off Calibrator	S3: Sample 2

7.3 Assay Steps

1. Ensure preparations from step 7.1 have been completed prior to pipetting.
2. Use the following steps in accordance with the quantitative/qualitative results desired:
3. Pipette, into the designated microwells as described in section 7.2, 100 µL of either:
    a. Calibrators (Cal A to Cal F) for Quantitative or
    b. Cut-off Calibrator (CC) for Qualitative

    And 100 µL of each of the following:

    c. Negative control (NC) and Positive Control (PC) and
    d. Diluted serum samples (S1, S2…)
4. Incubate for 60 minutes at 20-32°C/68-89.6°F.
5. Wash 3 times with 300 µL of 1x working wash buffer.
6. Pipette 100 µL of GAD65 Biotin solution (as described in chapter 7.1 above) into each well.
7. Incubate for 30 minutes at 20-32°C/68-89.6°F.
8. Wash 3 times with 300 µL of 1x working wash buffer.
9. Pipette 100 µL of conjugate into each well.
10. Incubate for 30 minutes at 20-32°C/68-89.6°F.
11. Wash 3 times with 300 µL of 1x working wash buffer.
12. Pipette 100 µl of TMB Substrate into each well.
13. Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from light.
14. Pipette 100 µl of stop solution into each well, using the same order as when the substrate was pipetted.
15. Incubate plate for a minimum of 5 minutes.
16. Agitate plate carefully for 5 seconds.
17. Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Calculation of Quantitative and Qualitative Results

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in **IU/mL (x-axis).** Log/lin coordinates and 4-Parameter Fit are recommended for best results. From the OD of each sample, read the corresponding antibody concentrations expressed in **IU/mL.**

Normal Range	Cut-off Range	Positive Range
< 20 IU/mL	20 - 30 IU/mL	>30 IU/mL

Example of a standard curve
Do not use this data for calculating assay results.

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0 IU/ml	0.129	5.8
25 IU/ml	0.380	4.8

75 IU/ml	0.743	5.5
125 IU/ml	1.134	3.1
250 IU/ml	1.612	1.1
500 IU/ml	2.484	2.8

Example calculation

Sample	Replicate (OD)	Mean (OD)	Result (IU/mL)
S1	0.454/0.442	0.448	54.39
S2	1.279/1.265	1.272	170.09

- Samples above the highest calibrator range should be reported as > Max and diluted as appropriate and re-assayed. Samples below the calibrator range should be reported as < Min.
- For lot-specific data, see enclosed quality control leaflet. Laboratories might perform an in-house quality control by using their own controls and/or internal pooled sera, as foreseen by national regulations.
- Each laboratory should establish its own normal range according to its own established procedures.
- If the values of the controls do not meet the criteria, the test is invalid and must be repeated.
- The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions, and washing methods.
- If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause, please contact ALPCO.
- **This kit is for research use only. It is not for use in diagnostic procedures.**

For **qualitative interpretation** read the optical density of the cut-off calibrator and the samples. Compare the sample´s OD with the OD of the cut-off calibrator. For qualitative interpretation, it is recommended to consider sera within range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative: $OD_{sample} < 0.8 \times OD_{cut\text{-}off}$
Equivocal: $0.8 \times OD_{cut\text{-}off} \leq OD_{sample} \leq 1.2 \times OD_{cut\text{-}off}$
Positive: $OD_{sample} > OD_{cut\text{-}off}$

9. Assay Characteristics

9.1 Summary

Sample material:	serum
Sample volume:	75 µl of sample diluted 1:4 with 1x working sample buffer
Total incubation time:	150 minutes at 20-32°C/68-89.6°F
Calibration range:	0 - 500 IU/ml
Analytical sensitivity:	9.25 IU/m
Storage:	at 2-8°C/35-46°F in original vials only
Number of determinations:	96 tests

10. Literature

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