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User's Manual

anti-Sulfate-3-Glucuronyl Paragloboside Autoantibodies ELISA Kit

REF **DEIA12536**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The CD anti-Sulfate-3-Glucuronyl Paragloboside Autoantibodies ELISA Kit is intended for the semi-quantitative determination of human IgM-autoantibodies directed against sulfate-3-glucuronyl para-globoside [SGPG] and sulfate-3-glucuronyl-lactosaminyl-paragloboside [SGLPG].

Principles of Testing

The anti-SGPG autoantibodies ELISA employs the quantitative enzymatically amplified sandwich-type immunoassay technique. Highly purified SGPG and SGLPG from bovine cauda equina have been precoated onto a microtiter plate. The Calibrator, Controls and patient sera are incubated for two hours in the microtiter wells and anti-SGPG autoantibodies present are bound by the immobilized bovine SGPG. After washing away any unbound substances, horseradish peroxidase (HRP) labeled antibodies against human IgM are added to the wells and incubated for another 2 hours. After a washing, the substrate solution containing tetramethylbenzidin (TMB) is added to the wells and incubated for 30 minutes. A blue coloration develops in proportion to the amount of anti-SGPG autoantibodies bound in the initial step. The color development is stopped by addition of the acidic stop solution (H_2SO_4) which turns the blue solution to yellow. The intensity of the color absorbance is measured in a microtiter plate reader at a wavelength of 450 nm. The absorbance measured is directly proportional to the concentration of anti-human SGPG autoantibodies.

Reagents And Materials Provided

1. Microtiter Plate, 96 wells precoated with bovine SGPG: 12 x 8-well strips with holder, Ready to use
2. Plate Sealer: 3 pieces
3. Wash Buffer Concentrate (10x) with preservatives: 1 bottle, 100 ml, Dilute with 900 ml of deionized water
4. Incubation Buffer with preservatives: 1 bottle, 100 ml, Ready to use
5. Calibrator1), Human serum with preservatives: 1 vial, Add 1 ml of Incubation Buffer
6. Control Low, Medium and High2, Human serum with preservatives: 3 vials, Add 1 ml of Incubation Buffer
7. Enzyme Label, Anti-IgM-HRP in a protein-based buffer; preservatives: 1 vial, 11 ml, Ready to use, Blue solution
8. TMB Substrate, TMB in Citrate buffer with Hydrogen Peroxide: 1 vial, 11 ml, Ready to use
9. Stop Solution, 0.25 M sulfuric acid: 1 vial, 11 ml, Ready to use, Corrosive agent

1) The Calibrator consists of a diluted positive serum which has been standardized to an internal established reference (see chapter standardization and cut-off).

2) Low, Medium and High Control contain lot-specific amounts of anti-SGPG antibodies. Refer to the QC data sheet provided with the kit for the corresponding ratios.

Materials Required But Not Supplied

1. Precision pipettes with disposable tips: 2 µl, 100 µl and 1ml pipettes.

2. Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions.
3. 1000 ml cylinder for the dilution of the Wash Buffer Concentrate.
4. Microtiter plate washer or squeeze bottle for Wash Buffer.
5. Microtiter plate rotator.
6. Microtiter plate reader for measurement of absorbance at 450 nm.

Storage

Unopened Reagents: Store at 2-8°C. Do not use past kit expiration date printed on the label.

Specimen Collection And Preparation

The procedure calls for <0.1 ml of blood or <50 µl of serum. Lipemic, hemolytic and icteric samples should not be used in this assay. Lipemic samples can be avoided by asking patients to fast for at least 12 hours prior to the sample being taken. Collect blood into plain tubes, avoid hemolysis, leave to clot for one hour at RT (18-28°C), centrifuge for 10 minutes at approximately 1000 x g at RT and collect the serum.

Store serum samples at ≤-20°C. Samples are stable for ≥1 year if stored at ≤-20°C. Avoid repeated freeze-thaw cycles. Frozen samples should be thawed and mixed thoroughly by gentle swirling or inversion prior to use.

Assay Procedure

Note: use refrigerated reagents in steps 3. to 9.

1. Dilute all patient samples 1:1000 with Incubation Buffer (e.g. 2 µl of serum + 2 ml of Incubation Buffer). Mix thoroughly by vortexing and leave diluted samples for 60 minutes at 18-28°C. Put samples for 10 minutes on ice prior to pipetting in step 4f.
2. Use a plate with enough 8-well strips to test the desired number of blank, calibrator, controls and samples. Remove excess strips from the holder and re-seal them together with the two desiccant bags without delay. Store refrigerated.
3. Wash the coated strips four times using at least 300 µl of wash buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

4a . Pipet 100 µl of Incubation Buffer (blank) in duplicate into wells A1+A2.

4b .Pipet 100 µl of Calibrator in duplicate into wells B1+B2

4c .Pipet 100 µl of Low Control in duplicate into wells C1+C2

4d . Pipet 100 µl of MediumControl in duplicate into wells D1+D2

4e . Pipet 100 µl of High Control in duplicate into wells E1+E2

4f . Pipet 100 µl of each diluted sample in duplicate into the subsequent wells.

5. Cover the plate with a plate sealer and incubate for 2 hours (± 5 min) at 2-8°C.
6. Remove and discard the plate sealer. Wash the plate four times using at least 300 µl of wash buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
7. Add 100 µl of Enzyme Label (blue solution) to all wells.

8. Cover the plate with a new plate sealer, and incubate for 2 hours (± 5 min) at 2-8°C.
9. Remove and discard the plate sealer. Wash the plate four times using at least 300 µl of wash buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

Important: Allow the TMB substrate solution to come to 18-28°C prior to using it in step 10.

10. Add 100 µl of TMB substrate solution to all wells.
11. Cover the plate and place the plate on a plate rotator set at 800-1000 rpm, protect the plate from direct light and incubate for 30 minutes (± 5 min) at 18-28°C.
12. Add 100 µl of stop solution to all wells. Remove air bubbles with a pipette tip. Proceed to step 13 within 30 minutes.
13. Read the absorbance at 450 nm in a microtiter plate reader.

Quality Control

A thorough understanding of this instruction for use (IFU) is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following this IFU.

CD strongly recommends testing Blank, Calibrator, Control and samples in duplicate.

Since there is no control serum for anti-SGPG antibodies commercially available, we recommend using a positive serum pool for internal quality controls.

All controls must fall within established confidence limits. The confidence limits for the Controls are lot-specific and printed on the additional data sheet.

The reproducibility of the calibrator and control values should be within established limits of laboratory acceptability. If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) ELISA reader settings iii) expiration dates of reagents iv) storage and incubation conditions v) TMB Substrate Solution should be colorless vi) purity of water.

Calculation

Calibrator: Record the absorbance at 450 nm (OD450) and subtract the averaged blank value. Average the duplicate values. The Ratio of the calibrator is set to a value of 1 (divided by itself).

Samples and Controls: Record the absorbance at 450 nm (OD450) for each sample and control well and subtract the averaged blank value. Average the duplicate values. Calculate the Ratio from the averaged sample absorbance to the averaged absorbance of the Calibrator.

Figure 1:

$$\text{Ratio} = \frac{\text{meannet OD450 of sample}}{\text{meannet OD450 of calibrator}}$$

Note: The results presented in Table below are examples. Calibrator and Controls must be used in each individual assay.

Table 1:

	OD_{450nm}	Mean OD_{450nm}	Mean Ratio
Blank	0.039		
Calibrator	0.378		
Calibrator	0.318	**0.348**	**1.00**
Control LOW	0.010		
Control LOW	0.012	0.011	**0.03**
Control MEDIUM	0.294		
Control MEDIUM	0.226	0.260	**0.75**
Control HIGH	0.834		
Control HIGH	0.731	0.783	**2.25**
Sample 1	0.953		
Sample 1	0.850	0.902	**2.6**
Sample 2	0.522		
Sample 2	0.537	0.530	**1.5**

Reference Values

Proposed Cut-off Ratio

The Background Ratio of anti-SGPG was determined using 200 blood samples from asymptomatic volunteer blood donors (49 female, 151 male at the age of 18 to 70 years) which were tested according to the assay procedure. Only 1/200 (0.5%) samples showed a Ratio of >1. Six sera showed a Ratio between 0.5 and 0.6, whereas 193/200 (96.5%) sera showed ratio values below 0.5.

14 anti-MAG positive sera were tested. Each serum showed a Ratio of 2.4 or higher. The results are listed in Table below. Therefore we propose a cut-off Ratio of 1.

Additionally, in an external study including 223 sera from patients affected by a suspected neuropathy the above proposed cut-off was verified. 178 sera were tested with the reference method thin layer chromatography (TLC) described in the literature. 99/110 positive TLC sera were tested positive in the anti-SGPG ELISA. 66/68 TLC negative sera were tested negative in the ELISA. This results in a calculated sensitivity and specificity of the ELISA of 90.0% and 97.1%, respectively.

Table 2:

	Ratio	
	Normal blood donors (n=200)	anti-MAG pos. sera (n=14)
Mean	**0.20**	**3.67**
SD	0.14	0.58
Min	0.07	2.36
Max	1.48	4.61
Mean+3SD	0.61	
Mean-3SD		1.92
95% percent.	0.48	

Detection Limit

Detection limit (LOB): <0.01 Ratio.

Detection limit (LOQ): <0.15 Ratio.

Linearity

151 %. 13 human serum samples containing high titer of anti-SGPG antibodies were diluted with Incubation Buffer 1:1000 to 1:128'000, left for one hour at 18-28°C and subsequently assayed according to the assay procedure. The ratio O/E (observed/expected) was calculated step by step. It is suggested that the relatively high deviation particularly at high antibody concentrations in the samples is due to antibody aggregations. In general, pathological sera show strongly elevated titer of autoantibodies therefore it has no influence to the positive/negative discrimination.

Table 4:

Sample Type	Range [min – max]	Mean [Observed/Expected]
Serum 4HF	100-114%	112%
Serum 5K	169-200%	175%
Serum 1C	86-133%	108%
Serum 2G	108-164%	141%
Serum 3GL	80-121%	110%
Serum 5	133-175%	161%
Serum 6	140-196%	180%
Serum 7	114-200%	157%
Serum 8	166-192%	175%
Serum 1	133-186%	171%
Serum 2	133-183%	161%
Serum 3	120-192%	164%
Serum 4	165-200%	185%
Mean		**154%**

Reproducibility

Intra-Assay Precision (Within-Run): 4.9%. The intra-assay precision was calculated from the results of 20 pairs of values from four human sera obtained in a single run.

The intra-assay precision: 3.3%-7.6%.

Inter-Assay Precision (Run-to-Run): 10.0%. The inter-assay precision was calculated from the results of 20 pairs of values from 6 human sera obtained in 20 different runs.

The inter-assay precision: 6.2%-13.4%.

Precautions

SAFETY PRECAUTIONS

1. Both, Calibrator and Controls of this kit contain components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with good laboratory practices using appropriate precautions.
2. Substrate and Stop Solution: The Substrate Solution contains Tetramethylbenzidine, hydrogen peroxide and dimethylformamide. The Stop Solution contains sulfuric acid. Each of those reagents is irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. After contact with eyes or skin, wash immediately with plenty of water.
3. Unused solution should be disposed of according to local State and Federal regulations.

TECHNICAL PRECAUTIONS

Kit components

1. Read carefully the instructions prior to carrying out the test. Test performance will be adversely affected, if reagents are incorrectly diluted, modified or stored under conditions other than those as detailed in this instruction for use:
2. Residues in the microtiter plate wells result from the production process. They are removed in the washing step and do not affect the results.
3. Steps 3-9: Use cold (2-8°C) reagents for all these steps and keep them cold while pipetting.
4. Steps 3, 6, 9: Make sure that the wells are completely empty after the last washing cycle.
5. Step 9: Adjust TMB Substrate to room temperature (18-28°C) before using it.
6. Step 11: Shake microtiter plates during the incubation with substrate. Depending on the plate shaker, we recommend 400-600 rpm. The solution should be moved in the wells but must not spill over.
7. If an automated washer is used, "plate mode" should be chosen so that dispensing is performed sequentially on all strips before aspirating.
8. Components must not be used after the expiry date printed on the labels.
9. Do not mix different lots of reagents.

Every effort should be made to ensure that no cross contamination occurs between reagents, samples or between wells.

11. Microwells cannot be re-used.

Limitations

Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.