anti-GM1 Autoantibodies ELISA

with Enzyme Labels IgG and IgM

For research use only.
Not for use in diagnostic procedures.

EK-GM1-GM-U 96 wells

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BÜHLMANN Laboratories AG
Baselstrasse 55
4124 Schönenbuch, Switzerland
Tel.: +41 61 487 1212
Fax: +41 61 487 1234
info@buhlmannlabs.ch

ENGLISH

INTENDED USE

The assay anti-GM1 Autoantibodies ELISA is designed for the quantitative determination of IgG and/or IgM of auto-antibody isotype directed against GM1 in human serum using individual IgG- and IgM-conjugates.
For research use only.

PRINCIPLE OF THE ASSAY

The anti-GM1 Autoantibodies ELISA is based on the enzyme-immunometric assay technique. The wells of the provided microtiter plate are coated with gangliosides: GM1.
Calibrator, Controls, and sera are incubated in the microtiter wells and anti-GM1 auto-antibodies present in the samples bind to the immobilized GM1. After washing off unbound substances, the antibodies are detected with horseradish-peroxidase (HRP) labelled antibodies against human IgG and/or IgM. Following a second washing step in which unbound enzyme label is removed, a substrate solution containing tetramethyl-benzidine (TMB) is added. A blue colour develops in proportion to the amount of anti-GM1 auto-antibodies bound to the gangliosides, GM1. Colour development is stopped by adding an acidic stop solution (diluted sulphuric acid) which turns the blue solution into yellow. The intensity of the colour is measured at 450 nm.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Microtiter Plate precoated with GM1	12 x 8 wells	B-GM1-MP	Ready to use
Plate Sealer	3 pieces		
Wash Buffer Concentrate (10X) with preservatives	1 bottle 100 mL	B-GCO-WB	Dilute with 900 mL of deionized water
Incubation Buffer with preservatives	1 bottle 100 mL	B-GCO-IB	Ready to use
Calibrator Lyophilized with preservatives	1 vial	B-GCO-CA	Add 1.5 mL of Incubation Buffer
Negative, Low and Medium Control Lyophilized with preservatives	3 vials	B-GCO-CONSET	Add 1.5 mL of Incubation Buffer
Enzyme Label IgG Anti-human IgG Ab conjugated to HRP in a protein-base buffer with preservatives	1 vial 11 mL	B-GCO-ELG	Ready to use
Enzyme Label IgM Anti-human IgM Ab conjugated to HRP in a protein-base buffer with preservatives	1 vial 11 mL	B-GCO-ELM	Ready to use
TMB Substrate TMB in citrate buffer	1 vial 11 mL	B-TMB	Ready to use
Stop Solution 0.25 M sulfuric acid	1 vial 11 mL	B-STS	Ready to use **Corrosive agent**

Table 1

STORAGE AND SHELF LIFE OF REAGENTS

Sealed / Unopened Reagents	
All sealed/unopened kit components are stable at 2-8 °C until the expiration date printed on the labels.	
Opened / Reconstituted Reagents	
Microtiter Plate	Return unused strips immediately to the aluminum pouch containing the desiccant packs and reseal along the entire edge of zip-seal. Store for up to 4 months at 2-8 °C.
Diluted Wash Buffer	Store for up to 4 months at 2-8 °C.
Calibrator	Store for up to 1 month at 2-8 °C. **Do not freeze!**
Controls	
Incubation Buffer	Store at 2-8° C until expiration date printed on the labels.
Enzyme Label	
TMB Substrate	
Stop Solution	Store at 18-28 °C until expiration date printed on the labels.

Table 2

PRECAUTIONS

Safety Precautions

- Both, Calibrator (B-GCO-CA) and Controls (B-GCO-CONSET) 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.
- Stop Solution: The Stop Solution (B-STS) contains sulfuric acid (0.25 M). The reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothes. After contact with eyes or skin, wash immediately with plenty of water.
- Reagents: Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation / burns can occur.
- Unused solution should be disposed of according to local state and federal regulations.

Technical Precautions

- 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.
- Residues in the microtiter plate wells result from the production process. They are removed in the washing step (assay procedure step 3) and do not affect the results.
- Prepare reagents before starting the assay procedure. Reagents used in steps 3-9 must be cold (2-8 ˚C) and kept cold while pipetting and washing. Put the TMB Substrate at room temperature (18-28 ˚C).
- Steps 3-9: Use cold (2-8 °C) reagents for all these steps and keep them cold while pipetting. Recommendation: Prepare the Wash Buffer the evening before performing the assay and place it into the fridge overnight.
- Wash steps 3, 6 and 9: The wash steps are crucial for removing residues in the microtiter plate wells resulting from the production process (step 3) as well as any unbound auto-antibodies (steps 6 and 9).

→ Always perform the wash steps with cold (2-8 ˚C) Wash Buffer.
→ Make sure that all wells are completely empty after the last washing cycle.

- Step 9: Adjust TMB Substrate to room temperature (18-28 °C) before using it.
- Step 11: Shake the microtiter plates during the incubation with substrate. Depending on the orbital plate shaker, we recommend 400-600 rpm. The solution should move in the wells but must not spill over.
- If an automated washer is used, "plate mode" should be chosen so that dispensing is performed sequentially on all strips before aspirating.
- Components must not be used after the expiry date printed on the labels.
- 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.
- Microwells cannot be re-used.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes with disposable tips: 20 µL, 100 µL and 1000 µL pipettes.
- Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions.
- 1000 mL cylinder for the reconstitution of the wash buffer.
- Squeeze bottle for Wash Buffer or automatic microtiter plate washer.
- Blotting paper.
- Orbital shaker for microtiter plates.
- Microtiter plate reader for measurement of absorbance at 450 nm.

SPECIMEN COLLECTION AND STORAGE

- The procedure requires <0.1 mL of blood and <50 µL of serum, respectively.
- Lipemic, hemolytic and icteric samples should not be used in this assay.
- Collect blood into plain tubes (no anti-coagulant), avoid haemolysis, leave to clot for one hour, centrifuge for 10 minutes at approximately 1500 x g at room temperature (18-28 °C), collect the serum.
- We recommend freezing aliquots of samples if you need to store samples in order to avoid repeated freezing/thawing.
- Store serum samples at ≤ -20 °C up to 4 months. For long-term storage we recommend -70 °C (samples are stable for >1 year). Frozen samples should be thawed and vortexed thoroughly prior to use.

ASSAY PROCEDURE

1. Dilute all samples to be investigated 1:50 with cold Incubation Buffer. Use 30 µL of serum + 1470 µL of Incubation Buffer. Mix by vortexing and leave diluted samples and reconstituted calibrator and controls for 30 minutes at 2-8 °C prior to pipetting.
2. Prepare a plate-frame with the required number of strips to test the samples. Reseal the remaining strips in the foil pouch together with the desiccant packs immediately. Store refrigerated.

Note: Use cold reagents in steps 3 to 9.

3. Wash coated wells twice using at least 300 µL of cold! Wash Buffer per well. Empty wells and tap plate firmly onto blotting paper to remove remaining liquid completely.

Note: Immediately proceed to the next steps.

Detection of IgG-Isotype:

Note: We recommend testing Calibrator, Controls and samples in duplicates

4a. Calibrator: Pipet 100 µL of the Calibrator into the well A1 and A2 (refer to Figure 1).

4b. Controls: Pipet 100 µL of the Control Medium into well B1, and B2, Control Low into well C1 and C2 and Control Negative into the well D1 and D2 (refer to Figure 1).

4c. Serum: Pipet 100 µL of diluted serum 1 into the wells E1-E2 (refer to Figure 1).

4d. Serum: Pipet 100 µL of diluted serum 2 into the wells F1-F2 (refer to Figure 1).

4e. Pipet 100 µL of diluted sera x-y into the subsequent wells (refer to Figure 1).

Detection of IgM-Isotype

4f. Repeat step 4a-4e using the subsequent wells.

Sample incubation and washes

5. Cover the plate with a Plate Sealer and incubate for 2 hours ±5 minutes at 2-8 °C (do not shake the plate).
6. Remove Plate Sealer. Empty the wells and wash three times using at least 300 µL of cold Wash Buffer (2-8 °C) per well. Empty the wells and strike the plate firmly onto blotting paper in order to remove washing buffer completely.

Detection of IgG and IgM Isotypes

7. Add 100 µL of Enzyme Label IgG or IgM to the respective wells.

Incubation with Enzyme Labels, washes, detection

8. Cover the plate with a Plate Sealer and incubate for 2 hours ± 5 minutes at 2-8 °C (do not shake the plate).
9. Remove Plate Sealer. Empty the wells and wash three times using at least 300 µL of cold Wash Buffer (2-8 °C) per well. Empty the wells and strike the plate firmly onto blotting paper.

Note: Adjust TMB Substrate Solution to room temperature (18-28 °C).

10. Add 100 µL of TMB Substrate Solution to each well.
11. Cover plate with a Plate Sealer, incubate plate on an orbital plate shaker at 400-600 rpm for 30 ± 2 minutes at 18-28 °C. Protect the plate from direct light.
12. Add 100 µL of Stop Solution to all wells. Proceed to step 13 within 30 minutes.
13. Read absorbance at 450 nm in a microtiter plate reader.

STANDARDIZATION

The Calibrator included in this kit has been calibrated against internal reference material. It has been adjusted to 100 % Ratio.

RESULTS AND CALCULATION

Calculation of Results:

1. Record absorbance (OD) at 450 nm for each well (Calibrator, Controls and samples).
2. Average the duplicate Calibrator and Control values (if available).
3. Results are expressed as ratio of absorbance of samples and the (averaged) absorbance of the Calibrator.

$$\%\ \text{Ratio:}\ \frac{\text{absorbance of samples and Controls}}{\text{absorbance of Calibrator}} \times 100$$

Programs to calculate results as % Ratio are available on most microplate readers.

QUALITY CONTROL

A good understanding of this instruction for use is necessary to obtain reliable results. These will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following the instruction for use.
Since there is no control serum for anti-GM1 auto-antibodies commercially available, we recommend using a positive, and negative serum pool for internal quality control.
The Calibrator must be within the established OD range. All Controls must be within established confidence ranges (% Ratio). The confidence ranges of the Calibrator and Controls are lot-specific. Please refer to the QC data sheet delivered with this kit for confidence ranges.
Performance characteristics should be within established limits. If these characteristics are not in conformity with established limits and repetition excludes handling failures, check the following issues: i) Have all reagents, used in step 3-10, been kept at 2-8 °C? ii) accuracy of pipets, thermometers, and timers, iii) settings of ELISA washer and reader, iv) expiration date of the reagents v) storage and incubation conditions vi) colour of the TMB Substrate Solution (should be colourless) vii) purity of the water.

PERFORMANCE LIMITATIONS

- The anti-GM1 Autoantibodies ELISA has not been validated for plasmapheresis samples.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (Within-Run): 7.0 %. The intra-assay precision was calculated from results of 12 values of three IgM and IgG samples in a single run. The values are listed in table 3 as % Ratio as described in “results and calculation”.

Inter-Assay Precision (Run-to-Run): 11.1 %. The inter-assay precision has been determined by measuring three serum samples with IgG and three with IgM antibodies to GM1 in 20 different runs. The values are listed in table 4 as % Ratio as describe in “results and calculation”.

Detection Limit (LOB): 12 Incubation Buffer replicates were assayed in a single run. The detection limit expressed as the % Ratio of the calibrator was calculated to be ≤5 %.

Linearity: The linear range of the test system was assessed according to CLSI guideline EP06-A. The system is linear in the diagnostic relevant range between 20 and 100 % Ratio. Results above 100 % Ratio are assessed correct and can be diluted into the linear range by an additional 1:5/1:10 dilution.

Specificity: Different human serum samples containing specific anti-ganglioside IgM and/or IgG antibodies were incubated over night with the corresponding soluble antigen in different concentrations and subsequently tested in the anti-GM1 Autoantibodies ELISA according to the assay procedure. Specificity of the antibody binding was demonstrated by inhibition with the corresponding antigen at concentrations between 1 and 100 µg/mL (data not shown).

INTERFERING SUBSTANCES

No interference is detected with the following substances up to the following concentrations: Triglycerides (Intralipid®): 3000 mg/dL; conjugated bilirubin: 60 mg/dL; unconjugated bilirubin: 40 mg/dL and hemoglobin: 400 mg/dL.

APPENDIX I

TABLES

Microtiter plate set-up: IgG & IgM-conjugate

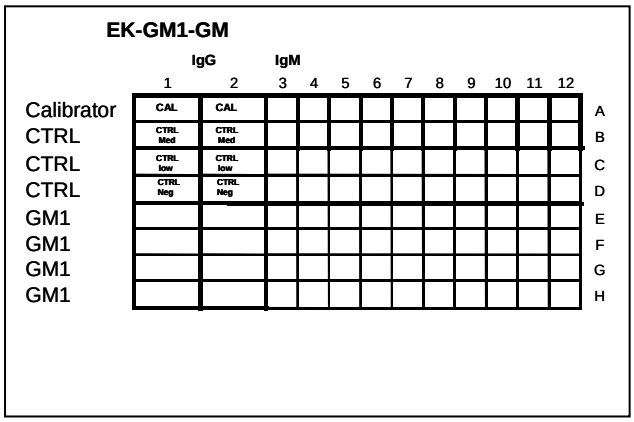


Figure 1

Intra-Assay Precision (Within-Run)

Anti-GM1	**Serum**	**Mean [% Ratio]**	**SD [% Ratio]**	**CV [%]**
Enzyme-Label IgG	1	116	11.1	9.6
	2	60	5.0	8.3
	3	150	11.2	7.5
Enzyme-Label IgM	1	78	5.3	6.8
	2	60	2.0	3.4
	3	105	6.6	6.3
Mean IgG/IgM				**7.0**

Table 3

Inter-Assay Precision (Run-to-Run)

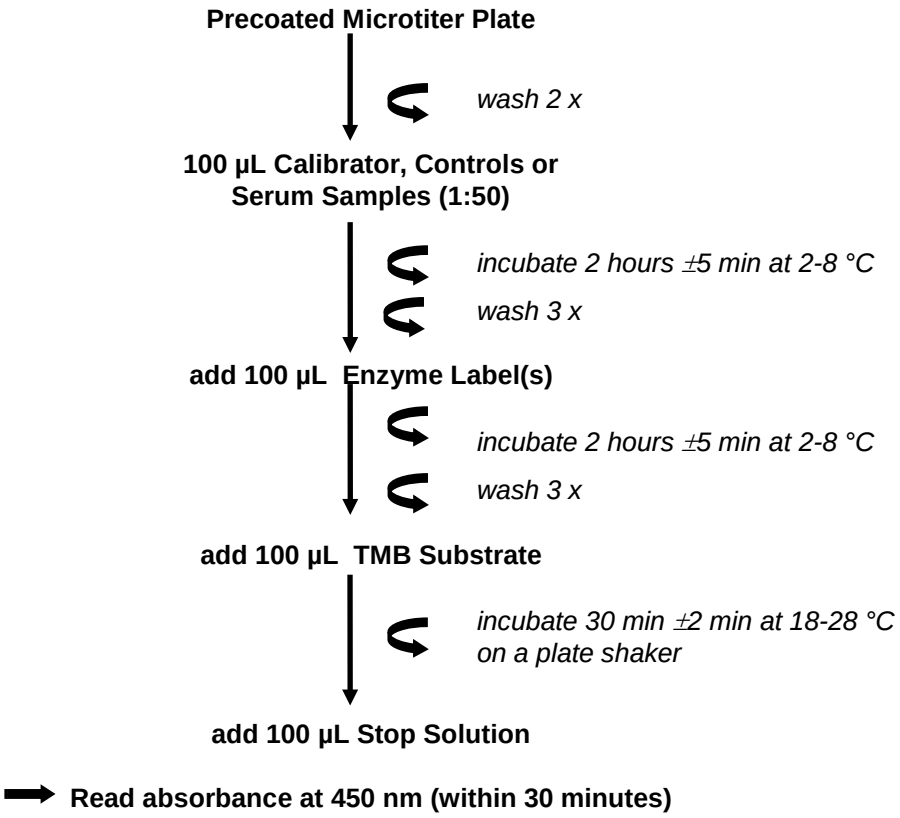
GM1	Serum	Mean [% Ratio]	SD [% Ratio]	CV [%]
Enzym-label IgG	1	134	11.3	8.5
	2	53	4.1	7.8
	3	132	31.4	23.9
Enzym-label IgM	1	92	8.3	9.1
	2	61	5.1	8.4
	3	95	8.5	8.9
Mean IgG/IgM				**11.1**

Table 4

APPENDIX II

NOTES

anti-GM1 Autoantibodies ELISA



TIME TO RESULT: 4.5 HOURS

APPENDIX V

SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Temperature limitation
MP	Microtiterplate
BUF WASH 10X	Wash Buffer Concentrate (10x)
BUF INC	Incubation Buffer
SOLN STOP	Stop Solution

Symbol	Explanation
CONTROL -	Negative Control
CONTROL L	Low Control
CONTROL M	Medium Control
CAL	Calibrator
EL IgG	Enzyme Label IgG
EL IgM	Enzyme Label IgM
SUBS TMB	TMB Substrate