BÜHLMANN
GanglioCombi™ MAG ELISA

with Enzyme Labels IgG/IgM Mix, IgG and IgM

Detection of anti-Ganglioside and -MAG auto-antibodies by ELISA

("MAG", GM1, GM2, GD1a, GD1b, and GQ1b)

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

EK-GCM-U 2 x 96 wells

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ENGLISH

INTENDED USE

BÜHLMANN GanglioCombi™ MAG ELISA, is an *in vitro* test intended to detect auto-antibodies against defined relevant neural antigens/epitopes in serum samples.
For research use only.

INTENDED APPLICATION

With regard to the 3 different Enzyme labels, the device components allow three application options:

1. Testing with the IgG/IgM Mix conjugate allows to screen for the presence of auto-antibodies.

2. Testing with individual IgG and/or IgM conjugates for auto-antibody isotype determination.

3. For laboratory work-up we suggest combining both procedures: sample screening using the Mix (option 1), followed by differentiation of Mix-positive samples by separate IgG and IgM enzyme labels (option 2).

PRINCIPLE OF THE ASSAY

BÜHLMANN GanglioCombi™ MAG ELISA is based on the enzyme-immunometric assay technique. The wells of the provided microtiter plate are coated with gangliosides: GM1, GM2, GD1a, GD1b and GQ1b as well as with a synthetic MAG (Myelin Associated Glycoprotein) “mimotope”. The MAG “mimotope” is a synthetic sulphated disaccharide. It mimics a MAG carbohydrate epitope, HNK-1, recognised by anti-MAG auto-antibodies.
Calibrator, controls, and sera are incubated in the microtiter wells and anti-ganglioside and/or -MAG auto-antibodies present in the samples bind to the immobilized gangliosides or MAG-analogue. After washing off unbound substances, the bonded auto-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 tetramethylbenzidine (TMB) is added. A blue colour develops in proportion to the amount of auto-antibodies bound to the immobilized gangliosides or MAG-analogue. 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 gangliosides and MAG analogue	2 x 12 x 8 wells	B-GCM-MP	Ready to use
Plate Sealer	6 pieces		
Wash Buffer Concentrate (10x) with preservatives	2 bottles 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/IgM Mix Anti-human IgG and IgM Ab conjugated to HRP in a protein-based buffer with preservatives	2 vials 11 mL each	B-GCO-ELGM	Ready to use
Enzyme Label IgG Anti-human IgG Ab conjugated to HRP in a protein-based 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-based buffer with preservatives	1 vial 11 mL	B-GCO-ELM	Ready to use
TMB Substrate TMB in citrate buffer	2 vials 11 mL	B-TMB	Ready to use
Stop Solution 0.25 M sulfuric acid	2 vials 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 aluminium 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 Labels	
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 the instructions carefully 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 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 the measurement of absorbance at 450 nm.

SPECIMEN COLLECTION AND STORAGE

- The procedure requires <0.1 mL of blood or <50 µL of **serum**, respectively.
- Refer to page 5 to learn about the interference of haemolysed, lipaemic or icteric samples.
- 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

You can choose between three basic options:

(1) Detection of IgG/IgM Mix-Isotypes: step 4a-4e and 7

(2) Detection of IgG and IgM Isotypes: step 4a‘-4f’and 7’

(3) Two-step approach: measurement of all samples with option 1 and post-measurement of the positive samples with separate enzyme conjugates (IgG and IgM) as option 2.

Note: Equilibrate TMB Substrate to room temperature (18-28 ˚C).

1. Dilute all samples to be investigated 1:50 with Incubation Buffer. Use 30 µL of serum + 1470 µL (cold: 2-8 °C!) Incubation Buffer. Mix by vortexing and leave diluted samples as well as reconstituted calibrator and controls for 30 minutes at 2-8 °C prior to pipetting (refer to steps 4a and b).
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.

Option 1: Detection of IgG/IgM Mix-Isotypes

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

4b. Controls: Pipet 100 µL of Medium Control into well B1, of Low Control into well A2 and of Negative Control into well B2 (refer to Figure 1A).

Note: If more than three strips per run are used, Calibrator and Controls can be tested in duplicates (see Figure 1A).

4c. Serum: Pipet 100 µL of diluted serum 1 into wells C1-H1 (refer to Figure 1A).

4d. Serum: Pipet 100 µL of diluted serum 2 into wells C2-H2 (refer to Figure 1A)

4e. Pipet 100 µL of diluted sera 3-24 into subsequent wells (refer to Figure 1A).

Option 2: Detection of IgG Isotypes

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

4b'. Controls: Pipet 100 µL of Medium Control into well B1, of Low Control into well A2 and of Negative Control into well B2 (refer to Figure 1B).

Note: If more than three strips per isotype are used, Calibrator and Controls can be tested in duplicates (see Figure 1B).

4c'. Serum: Pipet 100 µL of diluted serum 1 into wells C1-H1 (refer to Figure 1B).

4d'. Serum: Pipet 100 µL of diluted serum 2 into wells C2-H2 (refer to Figure 1B).

4e'. Pipet 100 µL of diluted sera 3-12 into subsequent wells.

Detection of IgM Isotypes.

4f'. Repeat steps 4a'-4e' using subsequent wells or a new microtiter plate if necessary (refer to Figure 1B).

For options 1 and 2: 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.

For option 1: Detection of IgG/IgM Mix-Isotype

7. Add 100 µL of Enzyme Label IgG/IgM Mix to the wells.

For option 2: Detection of IgG and IgM Isotypes

7'. Add 100 µL of Enzyme Label IgG or IgM to the respective wells (refer to Figure 1B).

For option 1 and 2: 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%**.**

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.

IgG/IgM Mix Isotypes

$$\%\ \text{Ratio} : \frac{\text{absorbance of samples or Controls}}{\text{absorbance of Calibrator}} \times 200$$

IgG and IgM Isotypes

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

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.
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) did all reagents, used in steps 3-9 have a temperature of 2-8 °C? ii) accuracy of the pipets, thermometers, and timers, iii) settings of ELISA washer and reader, iv) expiration date of reagents v) storage and incubation conditions vi) colour of TMB Substrate Solution (should be colourless) vii) purity of the water.

PERFORMANCE LIMITATIONS

- The BÜHLMANN GanglioCombi™ MAG ELISA has not been validated for plasmapheresis samples.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (Within-Run): ≤14.2 % CV The intra-assay precision was determined by performing 12 assay repetitions (IgG/IgM mix, IgG and IgM detection) with two samples in a single run.

Inter-Assay Precision (Run-to-Run): ≤21 % CV. The inter-assay precision was determined for two samples measured in 20 independent runs (IgG/IgM mix, IgG and IgM detection).

Detection Limit (LoB): ≤10 % Ratio of the Calibrator. 12 Incubation Buffer replicates were assayed in a single run.

Detection Limit (LoQ): Reliable results can be expected within and also outside the relevant diagnostic range. Inter and intra- assay precision of samples between 4 to 222 % Ratio for IgG and IgM enzyme labels and between and 9 to 304 % Ratio for IgG/IgM mix enzyme was determined. A polynomial curve of the obtained values was below the upper limit of 15 % and 20 % CV for intra- and inter-assay precision, respectively.

Specificity: Different human serum samples containing specific anti-ganglioside IgM and/or IgG auto-antibodies were incubated over night with the corresponding soluble antigen in different concentrations and subsequently tested in the BÜHLMANN GanglioCombi™ MAG ELISA according to the assay procedure. Specificity of the auto-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®): 2000 mg/dL; conjugated bilirubin: 60 mg/dL; unconjugated bilirubin: 40 mg/dL and hemoglobin: 400 mg/dL.

Microtiter plate set-up: IgG/IgM-Mix conjugate

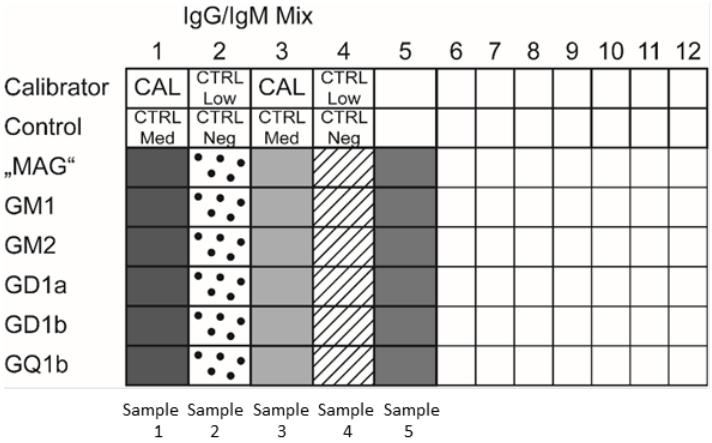


Figure 1A: ≤ 24 samples / Kit (2 MP / Kit)

Microtiter plate set-up: IgG & IgM conjugate

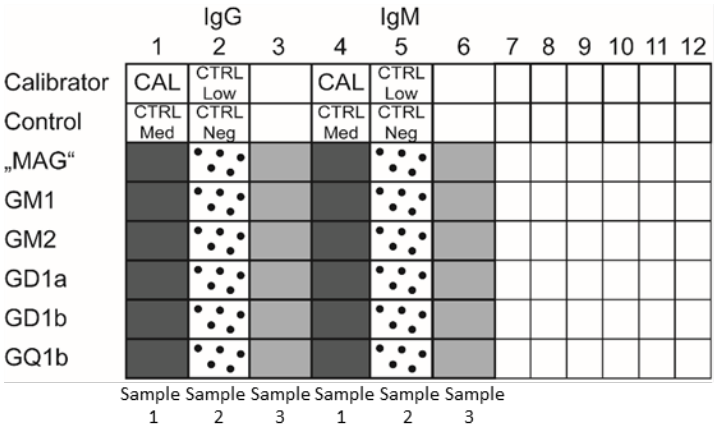
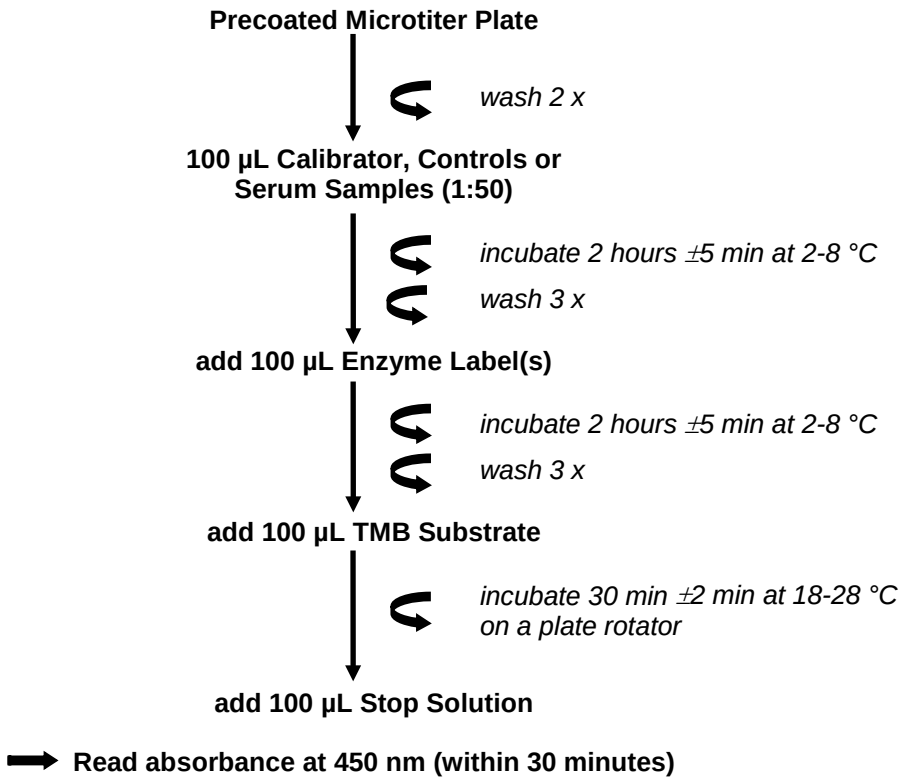


Figure 1B 2 profiles / samples, ≤ 12 samples / Kit (2 MP / Kit)

BÜHLMANN GanglioCombi™ MAG ELISA



TIME TO RESULT: 4.5 HOURS

APPENDIX III

SYMBOLS

Symbol	Explanation	Symbol	Explanation
	Use By	CONTROL -	Negative Control
REF	Catalogue number	CONTROL L	Low Control
LOT	Batch code	CONTROL M	Medium Control
	Contains sufficient for <n> tests	CAL	Calibrator
	Consult Instructions for Use-	EL IgG	Enzyme Label IgG
	Temperature limitation	EL IgM	Enzyme Label IgM
MP	Microtiterplate	EL MIX	Enzyme Label IgG/IgM Mix
BUF WASH 10X	Wash Buffer Concentrate (10x)	SUBS TMB	TMB Substrate
BUF INC	Incubation Buffer	SOLN STOP	Stop Solution

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