# BÜHLMANN GanglioCombi® MAG ELISA

## with enzyme labels IgG/IgM Mix, IgG and IgM

## Detection of anti-ganglioside and -MAG antibodies by ELISA

(HNK-1 (“MAG”), GM1, GT1a, GD1a, GD1b and GQ1b)

For *In Vitro* Diagnostic Use

EK-GCM 2 x 96 tests

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Version A1

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# ENGLISH

## INTENDED USE

The BÜHLMANN GanglioCombi® MAG ELISA is an *in vitro* diagnostic assay for the semi-quantitative determination of IgG and/or IgM antibodies against selected neural antigens/epitopes in serum samples. Assay results can be used to support the diagnosis of autoimmune peripheral neuropathies in conjunction with other clinical and laboratory findings.
For laboratory use only.

## INTENDED APPLICATION

The three enzyme labels, provided in the kit, enable three different testing algorithms:

1. Testing with the IgG/IgM conjugate mix (hereafter referred to as mix) allows to screen for the presence of anti-neural antibodies suggestive of an auto-immune neuropathy.
2. Testing with individual IgG and/or IgM conjugates allows antibody isotype determination.
3. For laboratory work-up initial sample screening using the mix (option 1), may be followed by differentiation of mix-positive samples using individual IgG and IgM conjugates (option 2), if required.

## PRINCIPLE OF THE ASSAY

The BÜHLMANN GanglioCombi® MAG ELISA allows the measurement of ganglioside and Myelin Associated Glycoprotein (MAG) antibodies in serum. The microtiter plate is coated with gangliosides: GM1, GT1a, GD1a, GD1b, GQ1b and and the chemically synthesized HNK-1 epitope of the MAG glycoprotein (ref. 1).
Patient sera, controls and calibrator are added to the wells of the microtiter plate. After 2 hours of incubation at 2 – 8°C and washing steps, detection antibodies (anti-IgG/IgM, anti-IgG, anti-IgM) conjugated to horseradish peroxidase (HRP) detect the anti-ganglioside and/or anti-MAG antibodies bound to the immobilized gangliosides or HNK-1 on the plate. After another 2 hours of incubation and further washing steps, the chromogenic HRP substrate, tetramethylbenzidine (TMB) is added (blue color formation) followed by a stopping reaction (change to yellow color). The absorption is measured at 450 nm.
The measured absorbance is proportional to the titer of antibodies present in a given sample. Antibody titers are expressed as % Ratios of the calibrator and can be assigned to titer categories (negative, grey zone, positive).

## REAGENTS SUPPLIED AND PREPARATION

| Reagents | Quantity | Code | Reconstitution |
|---|---|---|---|
| **Microtiter Plate** precoated with gangliosides and HNK-1 | 2 x12 x 8 well strips with frame | B-GCM-MP | Ready to use |
| **Plate Sealer** | 6 pieces | | |
| **Wash Buffer Concentrate (10x)** with preservatives | 2 bottles x 100 mL | B-GCO-WB | Dilute with 900 mL of deionized water |
| **Incubation Buffer** with preservatives | 1 bottle x 100 mL | B-GCO-IB | Ready to use |
| **Calibrator** lyophilized with preservatives | 1 vial | B-GCO-CA | Add 1.5 mL of Incubation Buffer |
| **Control Negative, Low and Medium** [1] 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 antibody conjugated to HRP in a buffer matrix with preservatives | 2 vials x 11 mL | B-GCO-ELGM | Ready to use |
| **Enzyme Label IgG** anti-human IgG antibody conjugated to HRP in a buffer matrix with preservatives | 1 vial x 11 mL | B-GCO-ELG | Ready to use |
| **Enzyme Label IgM** anti-human IgM antibody conjugated to HRP in a buffer matrix with preservatives | 1 vial x 11 mL | B-GCO-ELM | Ready to use |
| **TMB Substrate** TMB in citrate buffer | 2 vials x 11 mL | B-TMB | Ready to use |
| **Stop Solution** 0.25 M sulfuric acid | 2 vials x 11 mL | B-STS | Ready to use **Corrosive agent** |

Table 1

[1] The controls contain lot specific levels of anti-GM1 antibodies. Refer to the additional QC data sheet for actual mean OD and % Ratio.

## STORAGE AND SHELF LIFE OF REAGENTS

| **Sealed/ unopened reagents** | |
|---|---|
| Store at 2-8 °C. Do not use the reagents beyond the expiration date printed on the labels. | |
| **Opened/ reconstituted reagents** | |
| Microtiter Plate | Return unused strips immediately to the foil pouch containing the desiccant packs and reseal along the entire edge of zip-seal.<br>Store for up to 6 months at 2-8 °C. |
| Diluted Wash Buffer | Store for up to 6 months at 2-8 °C. |
| Incubation Buffer | |
| Enzyme Labels | |
| TMB Substrate | |
| Calibrator | |
| Controls | |
| Stop Solution | Store for up to 6 months at 18-28 °C. |

Table 2

## MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes with disposable tips: 10 µL, 20 µL, 100 µL and 1000 µL pipettes
- Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions
- 1000 mL cylinder for the dilution of the wash buffer
- Microtiter plate washer
- Blotting paper
- Microtiter plate shaker
- Microtiter plate reader for measurement of absorbance at 450 nm

## WARNINGS AND PRECAUTIONS

### Safety precautions

- The calibrator and controls of this kit contain components of human origin. Although tested and found negative for HBV, HCV and HIV1/2, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practices (GLP) using appropriate precautions.
- This kit contains components classified in accordance with the Regulation (EC) No. 1272/2008:
  - The stop solution contains sulfuric acid (conc. 2.5 – 5%), thus the reagents may cause skin irritation (H315), serious eye irritation (H319), and may be corrosive to metals (H290).
  - The calibrator, controls and enzyme labels contain 2-methyl-4-isothiazolin-3-one hydrochloride (conc. ≥ 0.0015%), thus the reagents may cause allergic skin reactions (H317).
  - The incubation buffer and wash buffer contain gentamicin sulphate, thus, the reagents may cause an allergic skin reaction (H317).
- 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.
- Reagents and chemicals have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

### 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.

### ELISA procedure

#### Temperature of reagents

- Prepare reagents before starting the assay procedure. Steps 3-9: Reagents used in steps 3-9 must be cold (2-8 °C) and kept cold while pipetting and washing. Recommendation: Prepare the wash buffer the day before performing the assay and place it into the fridge overnight.
- Perform all wash steps with cold (2-8 ˚C) wash buffer.
- Adjust TMB substrate and stop solution to room temperature (18-28 °C) at the start of the assay procedure.

#### Washing steps

- Wash steps 3, 6 and 9 are crucial to remove residues resulted from the production process and/or potentially unbound antibodies in the wells.
- An automated washer operating in “plate mode” is strongly recommended, i.e. each process step (dispense / aspiration) is carried out on all of the strips, sequentially, before the instrument continues with the next washing cycle.
- Make sure that all wells are completely empty after the last washing cycle.

#### Substrate incubation

- Step 11: Shake the microtiter plates during incubation with substrate. Depending on the model of the plate shaker we recommend 400-600 rpm. The solution should move in the wells but must not spill over.

### Kit components

- 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.

## SPECIMEN COLLECTION AND STORAGE

The procedure requires <0.1 mL of blood or <50 µL of serum, respectively.

Collect blood into plain venipuncture tubes without any additives and avoid hemolysis. Perform serum preparation according to manufacturer’s instructions. Decant the serum.

Serum samples can be stored at 2-8 °C for up to eight weeks, at 28 °C for up to one week and at ≤-20 °C for 16 weeks. Frozen samples should be thawed and mixed thoroughly by gentle swirling or inversion prior to use.
We recommend preparing aliquots of serum samples before freezing in order to avoid repeated freeze/thaw cycles.

## ASSAY PROCEDURE

**There are two options:**

(1) Detection of mix-isotypes (IgG and IgM): add enzyme label mix in step 7

(2) Detection of IgG or IgM isotypes: add either enzyme label IgG or enzyme label IgM in step 7

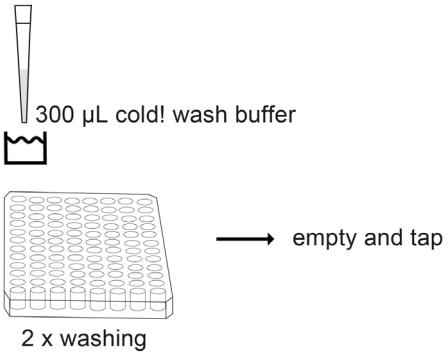
*Note: Adjust TMB substrate solution to room temperature (18-28 °C).*

1. Dilute samples 1:50 with incubation buffer. Use e.g. 20 µL of serum + 980 µL of cold! (2-8 °C) incubation buffer. Mix thoroughly by vortexing and leave diluted samples as well as reconstituted calibrator and controls at 2-8 °C for 30 minutes prior to pipetting (refer to step 4a and b).

2. Prepare a plate-frame with sufficient strips to test the required number of calibrators, controls, and samples. Remove excess strips from the frame and reseal it in the foil pouch together with the desiccant packs without delay. Store refrigerated.

*Note: Use cold reagents in steps 3 to 9.*

3. Wash the wells twice using at least 300 µL of cold! (2-8 °C) wash buffer per well. Empty the wells and tap the plate firmly onto blotting paper to remove remaining liquid completely.




*Note: Immediately proceed to the next steps.*

4a. Pipet 100 µL of calibrator into the well A1 (refer to figure 1A for option 1 or figure 1B for option 2).

4b. 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 or 1B).

*Note for option 1: If more than three strips per run are used, calibrator and controls can be tested in duplicates (see figure 1A).*

*Note for option 2: Calibrator and controls should be run separately for the IgG and IgM isotypes (see figure 1B).*

4c. Pipet 100 µL of diluted sample 1 into wells C1-H1 (refer to figure 1A or 1B).

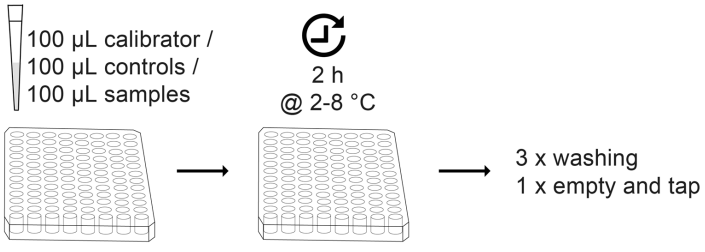
4d. Pipet 100 µL of diluted sample 2 into wells C2-H2 (refer to figure 1A or 1B).

4e. Pipet 100 µL of diluted samples 3-24 (for option 1) or 3-12 (for option 2) into subsequent wells (refer to figure 1A or 1B).

*Note for option 2: repeat the pipetting of samples 1-12 in the same order into the remaining wells for testing with the second isotype.*

5. Cover the plate with a plate sealer and incubate for 2 hours (±5 min) at 2-8 °C (do not shake the plate).

6. Remove the plate sealer. Empty the wells and wash three times using at least 300 µL of cold! (2-8 °C) wash buffer per well. Empty the wells and tap the plate firmly onto blotting paper in order to remove wash buffer completely.




**For option 1: Detection of mix-isotype**

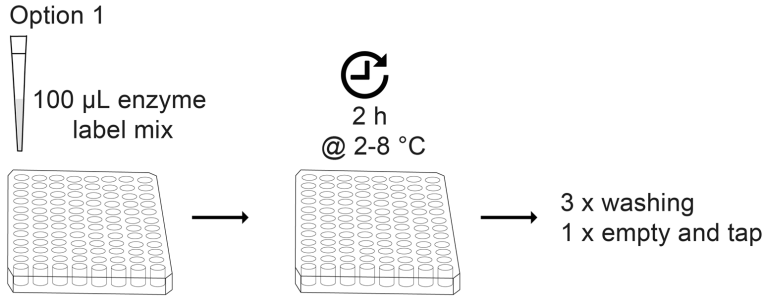
7. Add 100 µL of mix to the wells.

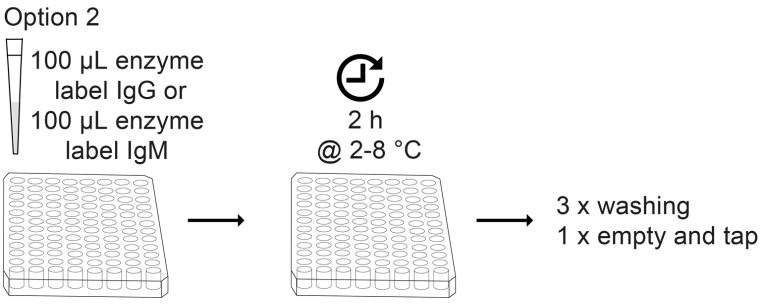
**For option 2: Detection of IgG or IgM isotypes**

7'. Add 100 µL of either enzyme label IgG or IgM to the respective wells (refer to figure 1B).

8. Cover the plate with a plate sealer and incubate for 2 hours (±5 min) at 2-8 °C (do not shake the plate).

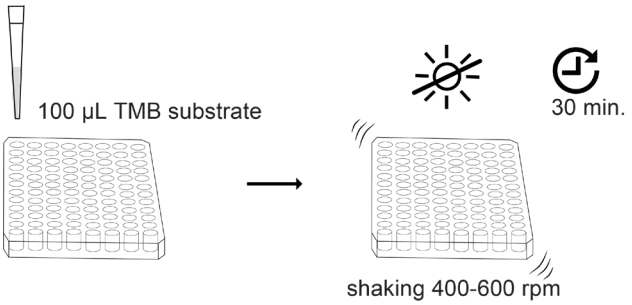
9. Remove the plate sealer. Empty the wells and wash three times using at least 300 µL of cold! (2-8 °C) wash buffer per well. Empty wells and tap the plate firmly onto blotting paper.



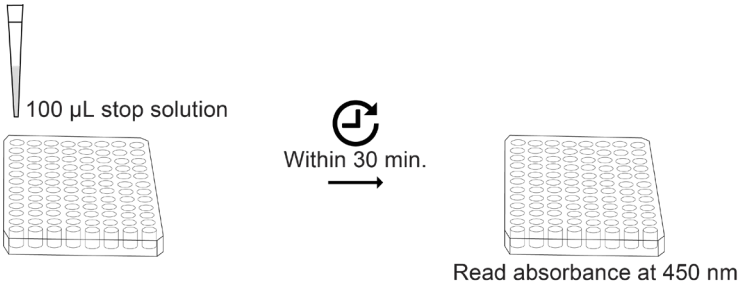

10. Add 100 µL of TMB substrate solution (equilibrated to room temperature) to each well.

11. Cover the plate with a plate sealer, protect the plate from light and incubate on a plate shaker set at 400-600 rpm, at 18-28 °C for 30 ±2 minutes.




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

Thorough understanding of this instruction for use is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques and accurately following this instruction for use.
The BÜHLMANN GanglioCombi® MAG ELISA kit comes with three controls: negative, low and medium control. The controls have assigned value ranges (% Ratio) indicated on the QC-data sheet supplied with each kit. The control measurements must be within the indicated value ranges to obtain valid results. In addition to kit controls, we recommend the use of serum pools for internal quality control.
A minimal $OD_{450nm}$ value of 1.2 is recommended for the calibrator.
Performance characteristics should be within established limits. If the performance of the assay does not meet the established limits and repetition has excluded errors in technique, check the following issues: i) temperature controlling (reagents used in step 3-9 kept at 2-8 °C) ii) accuracy of thermometers, pipetting and timing devices; iii) ELISA reader settings; iv) expiration dates of reagents; v) storage and incubation conditions; vi) color of TMB substrate solution (should be colorless); vii) purity of water; viii) aspiration and washing methods.

## STANDARDIZATION AND METROLOGICAL TRACEABILITY

There are no internationally or nationally recognized reference materials or reference measurement procedures for anti-ganglioside or -MAG antibodies in serum samples. The BÜHLMANN GanglioCombi® MAG ELISA is standardized against an internally established reference material. Calibrator values are assigned according to a value transfer protocol (ref. 2), to guarantee metrological traceability, and are indicated in arbitrary "% Ratio" units.
The 95% confidence interval of the combined uncertainty of product calibrators was determined to be 29.3% for IgG antibodies and 37.6% for IgM antibodies.

## CALCULATION OF TEST RESULTS

1. Record absorbance (OD) at 450 nm for each well (calibrator, controls and samples).
2. If multiple calibrator and control measurements were performed, average the values.

Results are expressed as Ratio of absorbance of samples and the (averaged) absorbance of the calibrator.

**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.

*Note: Results presented in tables 7 and 8 are examples and are provided for demonstration purposes only.*

## LIMITATIONS

- High % Ratio results (> 100%) for individual gangliosides may result in cross-reactivity with other gangliosides within the same sample. The cross-reactivity will typically show high inter-assay variation. The interpretation of results should therefore only be made together with an expert/specialist.
- Due to the poly-reactivity of auto-immune antibodies and differences in geographical prevalence, assay results should only be used to support the clinical interpretation of the neuropathy by an expert/specialist in combination with the patient's clinical picture (ref. 3).
- This test has not been validated for plasmapheresis.
- Intravenous immunoglobulins (IVIg) may affect test results.

## REFERENCE INTERVALS AND CUT-OFF

The reference interval of the BÜHLMANN GanglioCombi® MAG ELISA was established according to CLSI C28-A3 with 120 serum samples from self-declared healthy individuals. Distribution frequency of anti-ganglioside- and anti-MAG-antibodies in normal blood donors was classified in titer categories: negative (<30% Ratio), grey zone (30-50% Ratio) and positive (>50% Ratio). The results are summarized in table 9. The cut-off value for positivity is 50% Ratio.

## RESULT INTERPRETATION

| Antigen | IgG/IgM Mix | | |
|---|---|---|---|
| | Values (% Ratio) | | |
| | <30 | 30-50 | >50 |
| HNK-1 | Negative | Refer to annotation */** | Refer to annotation */** |
| GM1 | | Retest at a later time point | Positive |
| GT1a | | | |
| GD1a | | | |
| GD1b | | | |
| GQ1b | | | |

Table 3

| Antigen | IgG | | |
|---|---|---|---|
| | Values (% Ratio) | | |
| | <30 | 30-50 | >50 |
| HNK-1 | Negative | Refer to annotation * | Refer to annotation * |
| GM1 | | Retest at a later time point | Positive |
| GT1a | | | |
| GD1a | | | |
| GD1b | | | |
| GQ1b | | | |

Table 4

| Antigen | IgM | | |
|---|---|---|---|
| | Values (% Ratio) | | |
| | <30 | 30-50 | >50 |
| HNK-1 | Negative | Refer to annotation ** | Positive (refer to annotation **) |
| GM1 | | Retest at a later time point | Positive |
| GT1a | | | |
| GD1a | | | |
| GD1b | | | |
| GQ1b | | | |

Table 5

Test results should be interpreted in conjunction with information available from the clinical assessment of the patient and other diagnostic procedures.
* MAG neuropathy is commonly associated with presence of anti-MAG antibodies of the IgM isotype (ref. 4).
** Results between 30 and 50% (grey zone) or > 50% (positive) for HNK-1 obtained with the mix or enzyme label IgM may be re-tested with the anti-MAG Antibodies ELISA (EK-MAG).

## PERFORMANCE CHARACTERISTICS

### Method comparison

### BÜHLMANN GanglioCombi® MAG ELISA vs anti-MAG Antibodies ELISA

The method comparison study was performed according to the CLSI guideline EP09-A3 and EP12-A2. One hundred and twenty-two (122) samples were measured using 2 lots of BÜHLMANN GanglioCombi® MAG ELISA and 2 lots of anti-MAG Antibodies ELISA. Diagnostic (kappa) agreement, negative percent agreement and positive percent agreement were determined. The agreements are presented in table 10.

### Within-laboratory precision

**For anti-gangliosides: 5.7 – 13.2% CV**
**For anti-MAG: 14.4 – 36.5% CV**

Within-laboratory precision was established according to the CLSI guideline EP05-A3 using the standardized 20 days x 2 runs x 2 replicates study design. Three (3) pooled patient serum samples were tested. The results are summarized in table 11.

### Reproducibility

**For anti-gangliosides: 7.7 – 19.1% CV**
**For anti-MAG: 23.5 – 33.2% CV**

Reproducibility was established according to the CLSI guideline EP05-A3 using a 3 instrument/lot/operator x 5 days x 5 replicates study design. Three (3) pooled patient serum samples were tested. The results are summarized in table 12.

### Limit of blank (LoB) ≤ Limit of detection (LoD): ≤30% Ratio

The LoB and LoD was established according to the CLSI guideline EP17-A2 using the non-parametric analysis. The results are summarized in table 13.

### High dose hook effect

No limitation due to a high dose hook effect to the measuring range was observed.

### Cross-reactivity

No systematic cross-reactivity was observed for samples from patients with different auto-immune diseases (table 14) and from patients with other neurological disorders (table 15).

## CLINICAL PERFORMANCE

The clinical performance was assessed by summarizing analysis of peer-reviewed scientific literature. Six (6) studies addressed the clinical performance of the BÜHLMANN GanglioCombi® MAG ELISA in the diagnosis of autoimmune peripheral neuropathies (ref. 5-10). Results of analysis and study details are provided in table 6 and table 16, respectively.

| | |
|---|---|
| N peripheral neuropathy | 201 (102 pediatric GBS, 14 CIDP, 44 GBS, 41 anti-MAG neuropathy) |
| N controls | 493 (104 DC, 254 NC, 135 HC) |
| Sensitivity (95% CI) | 68.1 % (39.6 – 87.5 %) |
| Specificity (95% CI) | 88.0 % (72.3 – 95.3 %) |
| ROC AUC | 0.85 |

Table 6

GBS, Guillain-Barré-Syndrome; DC, Non-Neurological Disease Control; NC, Neurological Control; HC, Healthy Control; CIDP, Chronic Inflammatory Demyelinating Polyneuropathy; CI, confidence interval; ROC AUC, area under receiver operating characteristic curve

## INTERFERING SUBSTANCES

The susceptibility of the assay to oral and injectable pharmaceuticals, as well as to endogenous substances was assessed according to CLSI guideline EP07-A3. Bias in results ≥ ± 20% Ratio was considered interference.

No interference was detected with the following substances up to the listed concentrations: intravenous immunoglobulin (20 mg/mL), rituximab (3 mg/mL), cladribine (273 ng/mL), Interferon alpha-2a (49.5 ng/mL), gabapentin (26.7 µg/mL), ibuprofen (0.22 mg/mL), chlorambucil (1.96 µg/mL), prednisone (99 ng/mL), prednisolone (1.2 µg/mL), rheumatoid factor (2340 IU/mL), hemoglobin (10 mg/mL), hemolysate (10 mg/mL), triglyceride (15 mg/mL), conjugated bilirubin (20 µg/mL), unconjugated bilirubin (150 µg/mL).

## TABLES AND FIGURES

### Microtiter plate set-up: IgG/IgM-Mix label

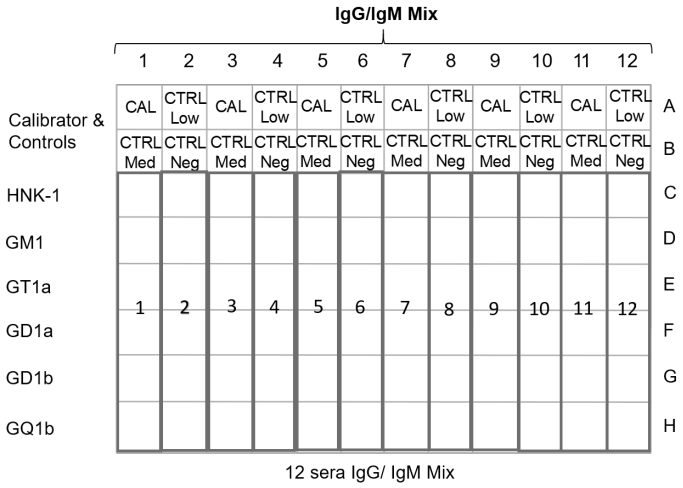



Figure 1A: ≤ 24 sera / Kit (2 MP / Kit)

### Microtiter plate set-up: IgG & IgM labels

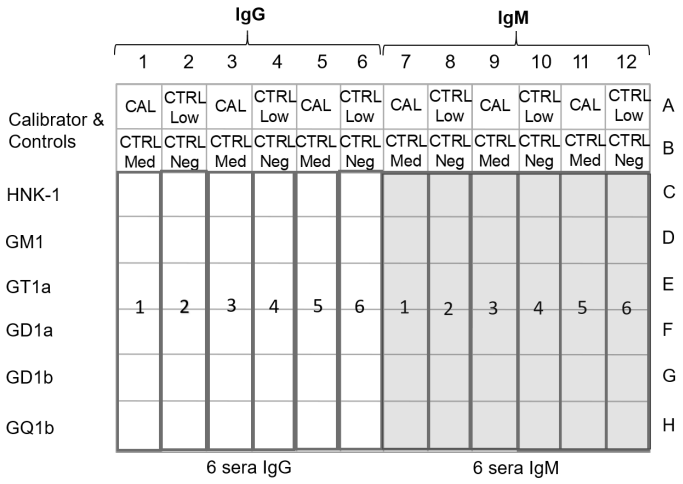



Figure 1B : 2 profiles / serum, ≤ 12 sera / Kit (2 MP / Kit)

### Example of results

#### A IgG/IgM-Mix label

| B-GCO-ELGM | Absorbance (OD450) | Ratio [%] |
|---|---|---|
| Calibrator | 2.179<br>2.477 | |
| Calibrator Avg. | 2.328 | 100 |
| Medium Control | 1.737<br>1.891 | |
| Medium Control Avg. | 1.814 | 78 |
| Low Control | 0.662<br>0.460 | |
| Low Control Avg. | 0.561 | 24 |
| Negative Control | 0.044<br>0.046 | |
| Negative Control Avg. | 0.045 | 2 |
| Sample 1<br>HNK-1 | 0.234 | 10 |
| Sample 1<br>GM1 | 0.543 | 23 |
| Sample 1<br>GT1a | 1.976 | 85 |
| Sample 1<br>GD1a | 0.621 | 27 |
| Sample 1<br>GD1b | 0.734 | 32 |
| Sample 1<br>GQ1b | 2.573 | 111 |

Table 7

#### B IgG & IgM labels

| Enzyme label | Absorbance (OD450) | | Ratio [%] | |
|---|---|---|---|---|
| B-GCO-ELG/<br>B-GCO-ELM | IgG | IgM | IgG | IgM |
| Calibrator | 2.488<br>2.446 | 2.411<br>2.201 | | |
| Calibrator Avg. | 2.467 | 2.306 | 100 | 100 |
| Medium Control | 1.879<br>1.987 | 1.734<br>1.818 | | |
| Medium Control Avg. | 1.933 | 1.776 | 78 | 77 |
| Low Control | 0.452<br>0.716 | 0.501<br>0.609 | | |
| Low Control Avg. | 0.584 | 0.555 | 24 | 24 |
| Negative Control | 0.045<br>0.037 | 0.048<br>0.042 | | |
| Negative Control. Avg. | 0.041 | 0.045 | 2 | 2 |
| Sample 1<br>HNK-1 | 0.423 | 0.621 | 17 | 27 |
| Sample 1<br>GM1 | 2.001 | 2.102 | 81 | 91 |
| Sample 1<br>GT1a | 0.521 | 0.237 | 21 | 10 |
| Sample 1<br>GD1a | 1.984 | 0.821 | 80 | 36 |
| Sample 1<br>GD1b | 0.473 | 1.923 | 19 | 83 |
| Sample 1<br>GQ1b | 0.094 | 0.911 | 4 | 40 |

Table 8

## TABLES AND FIGURES

### Reference interval

| Analyte | % normal blood donors in categories | | | Reference limit |
|---|---|---|---|---|
| | <30 %Ratio | 30 - 50 %Ratio | >50 %Ratio | (90% CI) |
| anti-MAG IgG | 96.7 | 2.5 | 0.8 | 25 (15.7 – 39.5) |
| anti-MAG IgM | 99.2 | 0.8 | 0.0 | 20 (18.6 - 28.4) |
| anti-MAG IgGM | 86.7 | 10.0 | 3.3 | 44 (34.8 – 52.9) |
| anti-GM1 IgG | 99.2 | 0.8 | 0.0 | 16 (13.0 – 29.8) |
| anti-GM1 IgM | 95.8 | 3.3 | 0.8 | 24 (14.3 – 40.3) |
| anti-GM1 IgGM | 95.0 | 4.2 | 0.8 | 34 (23.3 – 49.5) |
| anti-GT1a IgG | 90.0 | 6.7 | 3.3 | 44 (35.9 – 113.1) |
| anti-GT1a IgM | 97.5 | 2.5 | 0.0 | 16 (10.3 – 31.8) |
| anti-GT1a IgGM | 85.0 | 10.0 | 5.0 | 50 (42.4 – 140.3) |
| anti-GD1a IgG | 91.7 | 5.0 | 3.3 | 42 (26.2 – 108.2) |
| anti-GD1a IgM | 100.0 | 0.0 | 0.0 | 8 (6.6 – 12.4)$^{F}$<br>18 (6.6 – 24.3)$^{M}$ |
| anti-GD1a IgGM | 88.3 | 5.8 | 5.8 | 53 (35.0 – 118.7) |
| anti-GD1b IgG | 97.5 | 1.7 | 0.8 | 21 (14.5 – 33.0) |
| anti-GD1b IgM | 99.2 | 0.0 | 0.8 | 15 (6.3 – 15.5)$^{F}$<br>9 (6.4 – 54.7)$^{M}$ |
| anti-GD1b IgGM | 95.0 | 3.3 | 1.7 | 30 (22.3 – 71.6) |
| anti-GQ1b IgG | 97.5 | 2.5 | 0.0 | 24 (14.6 – 33.4) |
| anti-GQ1b IgM | 99.2 | 0.8 | 0.0 | 8 (6.2 – 17.8) |
| anti-GQ1b IgGM | 95.0 | 4.2 | 0.8 | 31 (23.1 – 46.7) |

*F* female subgroup. *M* male subgroup

Table 9

### Method comparison anti-MAG antibodies

| Descrip-tion | N | Kappa agreement | | NPA | | PPA | |
|---|---|---|---|---|---|---|---|
| | | Value | 95% CI | Value | 95% CI | Value | 95% CI |
| EK-GCM IgM vs. EK-MAG | 122 | 0.88 | 0.80 - 0.97 | 100.0% | 94.6% - 100.0% | 87.5% | 75.9%-94.8% |
| EK-GCM IgG/IgM Mix vs. EK-MAG | 122 | 0.87 | 0.78 - 0.96 | 97.0% | 89.5% - 99.6% | 89.3% | 78.1%-96.0% |

Table 10

NPA: Negative Percent Agreement
PPA: Positive Percent Agreement
CI: Confidence Interval

### Within-laboratory precision

| Sample Description | | | Within-Laboratory Precision | | | |
|---|---|---|---|---|---|---|
| Analyte | Enzyme Label (Isotype) | Expected Category [%Ratio] | N | Mean [%Ratio] | SD [%Ratio] | CV [%] |
| anti-GM1 Ab | IgM | 30-50 | 80 | 48 | 3.5 | 7.2 |
| | | >50 | 80 | 91 | 6.2 | 6.8 |
| | IgG | 30-50 | 80 | 40 | 5.1 | 12.9 |
| | | >50 | 80 | 106 | 13.1 | 12.4 |
| anti-GQ1b Ab | IgM | 30-50 | 80 | 45 | 2.6 | 5.7 |
| | | >50 | 80 | 85 | 6.7 | 7.8 |
| | IgG | 30-50 | 80 | 43 | 5.7 | 13.2 |
| | | >50 | 80 | 80 | 6.9 | 8.6 |
| anti-MAG Ab | IgM | 30-50 | 80 | 34 | 6.3 | 18.7 |
| | | >50 | 80 | 72 | 10.4 | 14.4 |
| | IgGM | 30-50 | 80 | 27 | 9.6 | 35.3 |
| | | >50 | 80 | 51 | 18.8 | 36.5 |

Table 11

### Reproducibility

| Sample Description | | | Reproducibility | | | |
|---|---|---|---|---|---|---|
| Analyte | Enzyme Label (Isotype) | Expected Category [%Ratio] | N | Mean [%Ratio] | SD [%Ratio] | CV [%] |
| anti-GM1 Ab | IgM | 30-50 | 75 | 51 | 4.9 | 9.7 |
| | | >50 | 75 | 94 | 7.2 | 7.7 |
| | IgG | 30-50 | 75 | 39 | 5.6 | 14.5 |
| | | >50 | 75 | 106 | 17.1 | 16.1 |
| anti-GQ1b Ab | IgM | 30-50 | 75 | 48 | 3.9 | 8.2 |
| | | >50 | 75 | 92 | 9.9 | 10.7 |
| | IgG | 30-50 | 75 | 42 | 8.1 | 19.1 |
| | | >50 | 75 | 78 | 12.0 | 15.4 |
| anti-MAG Ab | IgM | 30-50 | 75 | 43 | 14.3 | 33.2 |
| | | >50 | 75 | 98 | 23.1 | 23.5 |
| | IgGM | 30-50 | 75 | 42 | 10.6 | 25.0 |
| | | >50 | 75 | 97 | 27.2 | 28.0 |

Table 12

### LoD and LoB

| Analyte | LoB [% Ratio] | LoD [% Ratio] |
|---|---|---|
| Anti-GM1 IgM Ab | 5 | 21 |
| Anti-GM1 IgG Ab | 6 | 15 |
| Anti-MAG IgM Ab | 12 | 26 |
| Anti-MAG IgG/IgM Mix Ab | 14 | 27 |
| Anti-GQ1b IgM Ab | 3 | 17 |
| Anti-GQ1b IgG Ab | 8 | 18 |

Table 13

### Cross-reactivity

| Assigned antibody | Diagnose | # |
|---|---|---|
| Anti-neutrophil cytoplasmatic antibody (ANCA) | Vasculitis | 3 |
| | Others (ANCA positive denoted samples) | 10 |
| Anti-nuclear antibodies (ANA) | Systemic lupus erythematosus | 5 |
| | Rheumatoid arthritis | 9 |
| | Sjogren syndrome | 6 |
| | Others (ANA positive denoted samples) | 3 |
| Anti-thyroglobulin antibodies (anti-Tg) | Autoimmune thyroiditis | 5 |
| Anti-ribonucleoprotein antibodies | Mixed connective tissue disease | 1 |
| Anti-GQ1b, anti-GM1, anti-GD1b | Autoimmune peripheral neuropathy | 1 |
| Anti-acetyl-choline receptor antibodies and anti-muscle-specific tyrosine kinase | Myasthenia gravis | 7 |

Table 14

| Peripheral neuropathies | # |
|---|---|
| Alcoholic | 1 |
| Diabetic | 5 |
| **Peripheral neuropathy mimicking disorders** | **#** |
| Amyotrophic Lateral Sclerosis (ALS) | 15 |
| Sarcoidosis | 4 |
| Waldenstrom Macroglobulinemia (WM) | 4 |
| Chagas Disease | 5 |

Table 15

## TABLES AND FIGURES

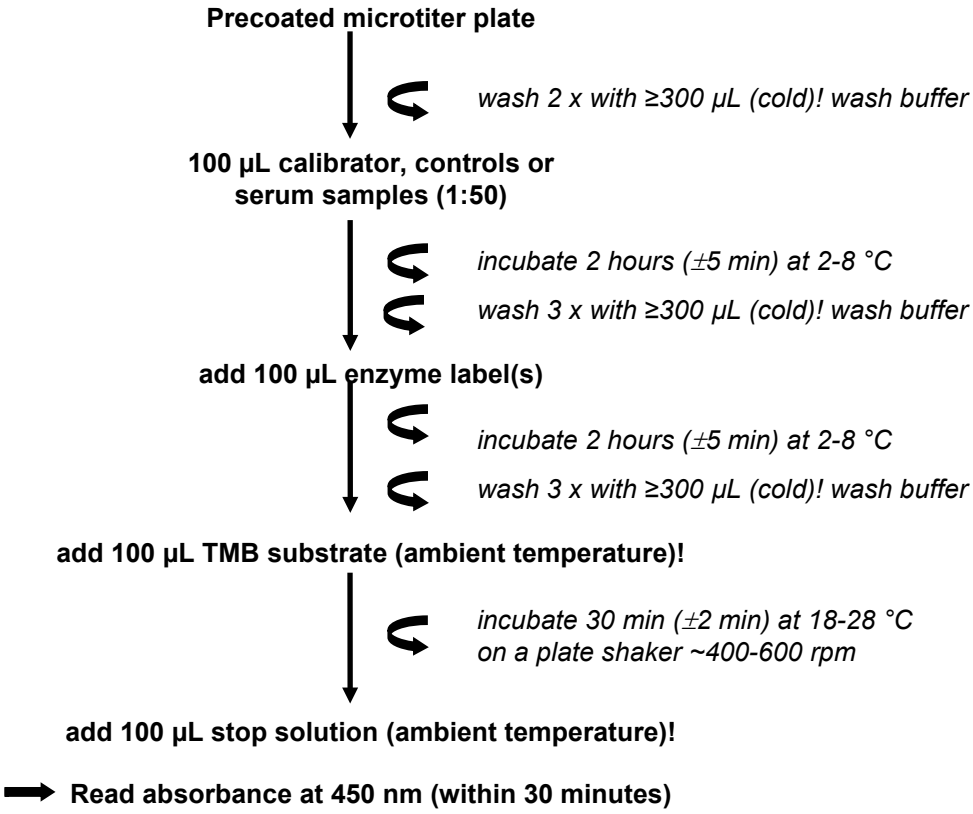
### Clinical performance

| Study | Positive controls (Cases) | Negative controls | Epitope | Sensi-tivity | Speci-ficity |
|---|---|---|---|---|---|
| Hashemilar et al., 2014 | Pediatric GBS (n = 45) | DC (n = 35) | GM1 | 0.51 | 0.89 |
| | | | GQ1b | 0.56 | 0.74 |
| Sharma et al., 2011 | Pediatric GBS (n = 57) | NC (n = 42) | GM1 | 0.82 | 0.33 |
| | | DC (n = 35) | | | 0.83 |
| Khandelwal et al., 2006 | GBS (n = 13) | HC (n = 19) | GM1 | 0.31 | 0.74 |
| Uetz-von Allmen et al., 1998 | GBS, CIDP (n = 19, 14) | NC (n = 100) | GM1 | 0.30 | 0.93 |
| | | HC (n = 110) | | | 0.95 |
| Spatola et al., 2016 | GBS (MFS) (n = 12) | DC (n = 34) | GQ1b | 0.92 | 0.97 |
| Delmont et al., 2019 | MAG-neuropathy (n = 41) | NC (n = 112)<br>HC (n = 6) | HNK-1 (MAG) | 0.98 | 0.99 |

Table 16

GBS, Guillain-Barré-Syndrome; DC, Non-Neurological Disease Control; NC, Neurological Control; HC, Healthy Control; MFS, Miller Fisher Syndrome; CIDP, Chronic Inflammatory Demyelinating Polyneuropathy

# BÜHLMANN GanglioCombi® MAG ELISA




**TIME TO RESULT: 4.5 HOURS**

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## CHANGELOG

| Date | Version | Change |
|---|---|---|
| 2023-08-17 | A1 | Change to the *Intended use* and product name<br>Removal of GM2 ganglioside and introduction of GT1a ganglioside<br>Rewording of the *Principle of the assay* with titer categories negative, grey zone, positive<br>New in use stabilities of reagents<br>Update to chapter *Warnings and Precautions*<br>Revision of chapters *Specimen collection and storage, Assay Procedure,* and *Standardization and metrological traceability*<br>Rewording of chapter *Quality Control*<br>Update to chapter *Limitations*<br>Revision of chapters *Reference Intervals and cut-off, Performance characteristics* and *Interfering substances,*<br>Introduction of chapter *Clinical Performance*<br>Revision of chapters *References* and *Symbols*<br>Inclusion of notified body number to CE-mark – conformity assessment procedure according to IVDR 2017/746 |

## INCIDENT REPORTING IN EU MEMBER STATES

If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

## SHIPPING DAMAGE

Please notify your distributor, if this product was received damaged.

## SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1. In addition, the following symbols and signs are used:

| Symbol | Explanation |
|---|---|
| MP | Microtiter Plate |
| BUF WASH 10X | Wash Buffer concentrate (10x) |
| BUF INC | Incubation Buffer |
| CAL | Calibrator |
| CONTROL - | Control Negative |
| CONTROL L | Control Low |
| CONTROL M | Control Medium |
| EL IgG | Enzyme Label IgG |
| EL IgM | Enzyme Label IgM |
| EL MIX | Enzyme Label IgG/IgM Mix |
| SUBS TMB | TMB Substrate |
| SOLN STOP | Stop Solution |
|  | **EN**: electronic instruction for use available in different languages at:/ **BG**: електронни инструкции за употреба на различни езици на адрес:/ **CS**: elektronický návod k použití dostupný v různých jazycích na adrese:/ **DA**: elektronisk brugsanvisning på forskellige sprog på:/ **DE**: elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ **EL**: ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ **ES**: instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ **ET**: elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ **FR**: un mode d'emploi électronique disponible en différentes langues à l'adresse:/ **HU**: különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ **IT**: istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ **LT**: elektroninės naudojimo instrukcijos įvairiomis kalbomis:/ **LV**: dažādās valodās pieejama elektroniska lietošanas instrukcija:/ **NO**: elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ **PL**: elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ **PT**: instrução electrónica para utilização disponível em diferentes línguas em:/ **RO**: instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ **SK**: elektronický návod na použitie dostupný v rôznych jazykoch na:/ **SL**: elektronska navodila za uporabo so na voljo v različnih jezikih na:/ **SR**: elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:/ **SV**: elektronisk bruksanvisning på olika språk på följande adress:<br>**www.buhlmannlabs.ch/support/downloads/** |

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