# BÜHLMANN GanglioCombi™ *Light* ELISA

## with Enzyme Labels IgG and IgM

## Detection of anti-Ganglioside Autoantibodies by ELISA

(GM1, GD1b and GQ1b)

EK-GCL-GM-U 96 wells

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# ENGLISH

## INTENDED USE

The BÜHLMANN GanglioCombi™ *Light* ELISA is intended for the quantitative determination of human IgG and IgM-autoantibodies directed against GM1, GD1b and GQ1b (IgG and IgM) (1-8).

## PRINCIPLE OF THE ASSAY

BÜHLMANN GanglioCombi™ *Light* ELISA is based on the enzyme-immunometric assay technique. GM1, GD1b and GQ1b have been coated onto the wells of the microtiter plate (Figure 1). Calibrator, controls and sera are incubated for two hours in the microtiter wells and anti-ganglioside autoantibodies (Ab) present in the sample bind to the immobilized gangliosides. After washing off unbound substances, horseradish-peroxidase (HRP) labeled antibodies against human IgG and / or IgM are added to the wells and incubated for another two hours. Following a second washing step in which unbound antibody-enzyme reagent is removed, a substrate solution containing tetramethylbenzidine (TMB) is added to the wells. A blue color develops in proportion to the amount of anti-ganglioside autoantibodies bound to the microplate in the initial step. Color development is stopped by adding an acidic stop solution (diluted sulfuric acid) which turns the blue solution into yellow. The intensity of the color is measured at 450 nm.

The measured absorbance is proportional to the titer of anti-ganglioside antibodies present in a given sample. The titers of anti-ganglioside autoantibodies are expressed as % ratios of the calibrator and can be assigned to titer categories.

## REAGENTS SUPPLIED AND PREPARATION

| Reagents | Quantity | Code | Reconstitution |
|---|---|---|---|
| **Microtiter Plate** precoated with gangliosides | 12 x 8 wells | B-GCL-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-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 with $H_2O_2$ | 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 aluminium pouch containing the desiccant packs and reseal along the entire edge of the 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° until expiration date printed on the labels. |
| Enzyme Labels | |
| TMB Substrate | |
| Stop Solution | Store at 18-28°C. |

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.
- **Substrate and Stop Solution:** The Substrate Solution (B-TMB) contains Tetramethylbenzidine (TMB), hydrogen peroxide and dimethylformamide. The Stop Solution (B-STS) 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.
- Unused solution should be disposed of according to local State and Federal regulations.

TECHNICAL PRECAUTIONS

- **Kit components:** 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.
- **Steps 3-9:** Use cold (2-8°C) reagents for all these steps and keep them cold while pipetting.
  Recommendation: Prepare washbuffer and incubation Buffer in particular on the eve of using it in the assay and to place it in the fridge overnight.
- **Steps 3, 6, 9:** Make sure that the wells are completely empty after the last washing cycle.
- **Step 9:** Adjust TMB Substrate to room temperature (18-28°C) before using it.
  Recommendation: Take it out of fridge when starting the assay.
- **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.
- 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.
- Microtiter plate shaker.
- 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.
- Do not use grossly hemolyzed, lipemic or icteric samples.
- Collect blood into plain tubes (no anti-coagulant), avoid hemolysis, leave to clot for one hour, centrifuge for 10 minutes at approximately 1500 x g at room temperature (18-28°C), collect the serum.
- For storage purposes we recommend preparing aliquots of samples in order to avoid repeated freezing/thawing.
- Store serum samples at ≤ -20°C for up to 4 months. For long-term storage we recommend -70°C (samples are stable for >1 year). Frozen samples should be thawed and mixed thoroughly by vortexing prior to use.

## ASSAY PROCEDURE

1. **Dilute** all samples to investigate 1:50 with cold Incubation Buffer (e.g. 20 µl of serum + 980 µl of Incubation Buffer). Mix by vortexing and leave diluted samples and reconstituted calibrator and controls for 60 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 after usage**. Store refrigerated.

***Note: Use cold (2-8° C) reagents in steps 3 to 10.***

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

**Detection of IgG-Isotype:**

4a. Calibrator: Pipet 100 µl of **Calibrator** into the well A1 (see Figure 1).

4b. Controls: Pipet 100 µl of **Medium Control** into well B1, **Low Control** into well A2 and **Negative Control** into the well B2 (see Figure 1).

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

4c. Serum: Pipet 100 µl of **diluted serum 1** into the wells C1 - E1 (see Figure 1).

4d. Serum: Pipet 100 µl of **diluted serum 2** into the wells F1 – H1 (see Figure 1).

4e. Pipet 100 µl of **diluted sera 3-12** into the subsequent wells (see Figure 1).

**Detection of IgM-Isotype:**

5. Repeat step 4a – 4e using the subsequent wells.
6. Cover the plate with a Plate Sealer and **incubate** for 2 hours ± 5 minutes at 2-8°C (do not shake the plate).
7. Remove Plate Sealer. Empty wells and **wash** three times using at least 300 µl of **cold** Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper in order to remove washing buffer completely.
8. Add 100 µl of **Enzyme Label IgG or IgM** to the respective wells.
9. Cover the plate with a Plate Sealer and **incubate** for 2 hours ± 5 minutes at 2-8°C (do not shake the plate).
10. Remove Plate Sealer. Empty 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: Let TMB Substrate Solution adjust to room temperature (18-28°C).***

11. Add 100 µl of TMB Substrate Solution to each well.
12. Cover plate with a Plate Sealer, incubate plate on a **plate shaker** at 400-600 rpm for 30 ± 2 minutes at 18-28°C protecting the plate from direct light.
13. Add 100 µl of **Stop Solution** to all wells. Proceed to step 14 within 30 minutes.
14. 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. Results are expressed as ratio of absorbance of samples and the absorbance of the Calibrator

**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 microtiter plate readers.

***Note: The results presented in Table 3 are examples. Calibrator and Controls must be used in each individual assay.***

## 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-ganglioside antibodies commercially available, we recommend using a positive, and negative serum pool for internal quality control.

All controls must be within established confidence ranges (% ratio). The confidence ranges of the Controls are lot-specific and printed on the QC data sheet delivered with this kit.

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 the pipets, thermometers, and timers, iii) settings of ELISA washer and reader, iv) expiration date of the reagents

v) storage and incubation conditions vi) color of the TMB Substrate Solution (should be colorless) vii) purity of the water.

## PERFORMANCE CHARACTERISTICS

**Intra-Assay Precision (Within-Run): 3.5 %.** The intra-assay precision was calculated from results of 12 values of one IgM and IgG sample with autoantibodies to GD1b, GM1 and GQ1 in a single run. The assay was performed using the individual anti-IgG and anti-IgM Enzyme Labels (B-GCO-ELG, B-GCO-ELM). The values are listed in
Table 4 as % Ratio as described in “Results and Calculation”.

**Inter-Assay Precision (Run-to-Run): 12.9 %.** The inter-assay precision has been determined by measuring two serum samples with autoantibodies to GD1b, GM1 and GQ1 using the individual anti-IgG and anti-IgM Enzyme Labels (B-GCO-ELG, B-GCO-ELM) in 20 different runs. The values are listed in Table 5 as % Ratio as describe in “Results and Calculation”.

**Detection limit (LoB):** 12 Incubation Buffer replicates were assayed in a single run for anti-IgG and anti-IgM Enzyme Labels (B-GCO-ELG, B-GCO-ELM). The detection limit, expressed as the % Ratio of the calibrator was calculated to be 7.2% (mean +2 SD). The results are displayed in Table 6.

**Dilution Linearity:** Between 30 and 200 % ratio, dilutions of tested serum samples result in a continuous reduction of the % ratio.

**Specificity:** Different human serum samples containing specific anti-ganglioside IgM and/or IgG autoantibodies were incubated over night with the corresponding soluble antigen and subsequently tested in the BÜHLMANN GanglioCombi™ *Light* ELISA according to the assay procedure. A 50 % inactivation for a specific ganglioside autoantibody has been observed at an antigen concentration between 10 and 100 µg/ml of specific antigen (data not shown).

Figure 1 **Microtiter plate set-up individual IgG and IgM conjugates**

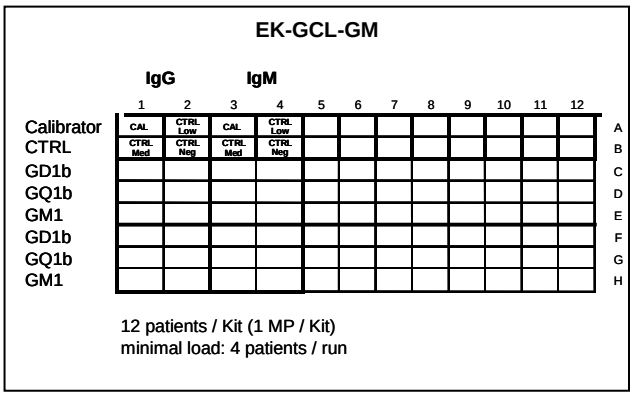


Table 3 **Example of Results**

| Enzyme label | Absorbance (OD450) | | Ratio [%] | |
|---|---|---|---|---|
| B-GCO-ELG/ B-GCO-ELM | IgG | IgM | IgG | IgM |
| **Calibrator** | 1.836 | 2.551 | **100** | **100** |
| **Me. Control** | 1.252 | 1.753 | **68** | **69** |
| **Low Control** | 0.571 | 0.940 | **31** | **37** |
| **Neg. Control.** | 0.056 | 0.097 | **3** | **4** |
| Sample 1 **GM1** | 0.171 | 3.814 | **9** | **150** |
| Sample 1 **GD1b** | 1.021 | 0.354 | **56** | **14** |
| Sample 1 **GQ1b** | 0.378 | 0.208 | **21** | **8** |

Table 4 **Intra-Assay Precision (Within-Run)**

| Ganglioside | Enzyme label | Mean [% Ratio] | SD [% Ratio] | CV [%] |
|---|---|---|---|---|
| GM1 | IgG | 81.6 | 4.0 | 4.9 |
| | IgM | 69.1 | 1.4 | 2.0 |
| GD1b | IgG | 53.9 | 1.8 | 3.3 |
| | IgM | 65.0 | 1.5 | 2.3 |
| GQ1b | IgG | 46.5 | 2.0 | 4.3 |
| | IgM | 102.0 | 4.1 | 4.0 |
| **Mean** | | | | **3.5** |

Table 5 **Inter-Assay Precision (Run-to-Run)**

| Ganglioside | Enzyme label | Mean [% Ratio] | SD [% Ratio] | CV [%] |
|---|---|---|---|---|
| GM1 | IgG | 24.4 | 4.3 | 17.4 |
| | | 37.5 | 7.3 | 19.5 |
| GM1 | IgM | 63.7 | 4.9 | 7.7 |
| | | 54.1 | 3.3 | 6.0 |
| GD1b | IgG | 23.4 | 4.7 | 20.0 |
| | | 37.1 | 6.9 | 18.6 |
| GD1b | IgM | 36.5 | 4.6 | 12.6 |
| | | 87.2 | 7.5 | 8.6 |
| GQ1b | IgG | 41.1 | 6.4 | 15.5 |
| | | 58.1 | 6.0 | 10.4 |
| GQ1b | IgM | 34.2 | 2.4 | 7.0 |
| | | 86.2 | 9.7 | 11.1 |
| **Mean** | | | | **12.9** |

Table 6 **Analytical Sensitivity**

| B-GCO-ELG - IgG | GM1 [% Ratio] | GD1b [% Ratio] | GQ1b [% Ratio] |
|---|---|---|---|
| mean<br>SD<br>Mean+2SD | 7.03<br>0.6<br>**8.22** | 6.81<br>1.35<br>**9.5** | 6.96<br>0.6<br>**8.17** |
| BGCO-ELM - IgM | | | |
| mean<br>SD<br>Mean+2SD | 4.32<br>0.84<br>**6.0** | 3.95<br>0.54<br>**5.03** | 4.24<br>1.01<br>**6.26** |
| **Mean** | | | **7.2** |

**APPENDIX II**

**REFERENCES**


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**APPENDIX III**

**SHORT PROTOCOL**

## BÜHLMANN GanglioCombi™ *Light* ELISA

**Precoated Microtiter Plate**

↓ ↺ *wash 2 x*

**100 µL Calibrator, Controls and Serum Samples (1:50)**

↓ ↺ *incubate 2 hours ±5 min at 2-8°C*

↺ *wash 3 x*

**add 100 µL Enzyme Label(s)**

↓ ↺ *incubate 2 hours ±5 min at 2-8°C*

↺ *wash 3 x*

**add 100 µL TMB Substrate**

↓ ↺ *incubate 30 min ±2 min at 18-28°C on a plate rotator*

**add 100 µL Stop Solution**

➡ **Read absorbance at 450 nm (within 30 minutes)**

**TIME TO RESULT: 4.5 HOURS**

## APPENDIX IV

## NOTES

**APPENDIX V**

**SYMBOLS**

| Symbol | Explanation |
|---|---|
|  | Use By<br>Verwendbar bis<br>Utiliser jusqu'au<br>Utilizzare entro<br>Fecha de caducidad |
| [REF] | Catalogue number<br>Bestellnummer<br>Référence du catalogue<br>Numero di catalogo<br>Número de catálogo |
| [LOT] | Batch code<br>Chargenbezeichnung<br>Code du lot<br>Codice del lotto<br>Codigo de lote |
|  | Temperature limitation<br>Zulässiger Temperaturbereich<br>Limites de température<br>Limiti di temperatura<br>Limite de temperatura |
| [MP] | Microtiterplate<br>Mikrotiterplatte<br>Plaque de microtitration<br>Microplaca<br>Microplaca |
| [BUF\|WASH\|10X] | Wash Buffer Concentrate (10x)<br>Waschpuffer-konzentrat (10x)<br>Concentré de tampon de lavage<br>Tampón de lavado concentrado (x10)<br>Tampón de lavado concentrado (x10) |
| [BUF\|INC] | Incubation Buffer<br>Inkubations-Puffer<br>Tampon d'incubation<br>Tampón de incubación<br>Tampón de incubación |

| Symbol | Explanation |
|---|---|
| [CONTROL\|-] | Negative Control<br>Negativkontrolle<br>Contrôle négatif<br>Control negativo<br>Control negativo |
| [CONTROL\|L] | Low Control<br>Kontrolle tief<br>Contrôle faible<br>Control bajo<br>Control bajo |
| [CONTROL\|M] | Medium Control<br>Kontrolle mittel<br>Contrôle moyen<br>Control medio<br>Control medio |
| [CAL] | Calibrator<br>Kalibrator<br>Calibrateur<br>Calibratore<br>Calibrador |
| [EL\|IgG] | Enzyme Label IgG<br>Enzymmarker-IgG<br>Marqueur enzymatique IgG<br>Marcato enzimatico IgG<br>Marcador enzimático IgG |
| [EL\|IgM] | Enzyme Label IgM<br>Enzymmarker-IgM<br>Marqueur enzymatique IgM<br>Marcato enzimatico IgM<br>Marcador enzimático IgM |
| [SUBS\|TMB] | TMB Substrate<br>TMB Substrat<br>Substrat TMB<br>Substrato di TMB<br>Substrato de TMB |
| [SOLN\|STOP] | Stop Solution<br>Stopp-Lösung<br>Solution stop<br>Soluzione stoppante<br>Solución de parada |