# BÜHLMANN

# Anti-GM1 Antibodies ELISA

## with enzyme labels IgG and IgM

## Detection of anti-ganglioside M1 antibodies by ELISA
(GM1)

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

EK-GM1-GM-U       96 tests

Release date: 2023-08-17
Version A2

# ENGLISH

## INTENDED USE

The anti-GM1 Antibodies ELISA is an assay for the semi-quantitative determination of IgG and/or IgM antibodies against the neural antigen/epitope GM1 in serum samples. For research use only. Not intended for use in diagnostic procedures.

## PRINCIPLE OF THE ASSAY

The anti-GM1 Antibodies ELISA allows the measurement of antibodies against GM1 (ganglioside M1) in serum. The microtiter plate is coated with gangliosides: GM1.

Serum samples, 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, anti-IgM) conjugated to horseradish peroxidase (HRP) detect the anti-GM1 antibodies bound to the immobilized GM1 ganglioside 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 anti-GM1 antibodies present in a given sample. Antibody titers are expressed as % Ratios of the calibrator.

## REAGENTS SUPPLIED AND PREPARATION

| Reagents | Quantity | Code | Reconstitution |
|---|---|---|---|
| **Microtiter Plate** precoated with GM1 | 12 x 8 well strips with frame | B-GM1-MP | Ready to use |
| **Plate Sealer** | 3 pieces | | |
| **Wash Buffer Concentrate (10X)** with preservatives | 1 bottle 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** 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 | 1 vial x 11 mL | B-TMB | Ready to use |
| **Stop Solution** 0.25 M sulfuric acid | 1 vial 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. 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 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

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

1. Dilute samples 1:50 with incubation buffer. Use e.g. 10 µL of serum + 490 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.

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 wells and tap the plate firmly onto blotting paper to remove remaining liquid completely.



300 µL cold! wash buffer

empty and tap

2 x washing
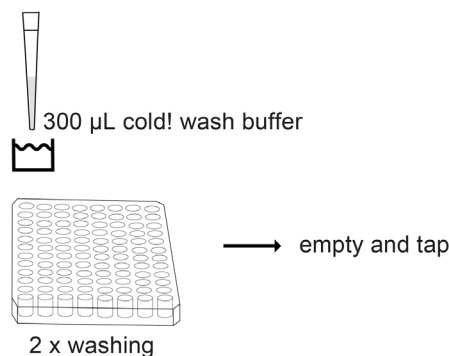
*Note: Immediately proceed to the next steps.*

### Detection of IgG-Isotype:

4a. Pipet 100 µL of calibrator into the well A1 (refer to figure 1a).

4b. Pipet 100 µL of medium control into well B1, of low control into well C1 and of negative control into well D1 (refer to figure 1a).

4c. Pipet 100 µL of diluted sample 1 into the wells E1 (refer to figure 1a).

4d. Pipet 100 µL of diluted sample 2 into the wells F1 (refer to figure 1a).

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

### Detection of IgM-Isotype

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

*Note: Calibrator and controls should be run separately for the IgG and IgM isotypes. We recommend testing calibrator, and controls in duplicates if more than 3 strips per run are used. See figure 1b for alternative pipetting scheme.*

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.

100 µL calibrator /
100 µL controls /
100 µL samples

2 h
@ 2-8 °C

3 x washing
1 x empty and tap
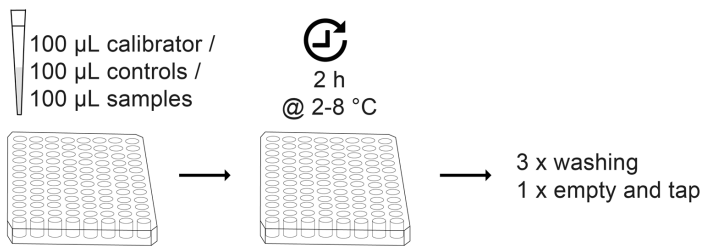
7. Add 100 µL of either <u>enzyme label IgG</u> or <u>IgM</u> to the respective wells (refer to figure 1).

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 the wells and tap the plate firmly onto blotting paper.



100 µL enzyme label IgG or
100 µL enzyme label IgM

2 h
@ 2-8 °C

3 x washing
1 x empty and tap
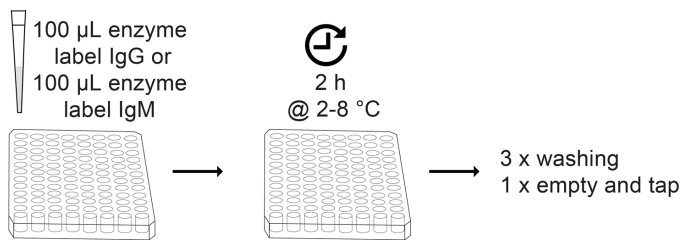
10. Add 100 µL of TMB substrate solution (equilibrated to room temperature) to each well.

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.



100 µL TMB substrate

30 min.

shaking 400-600 rpm
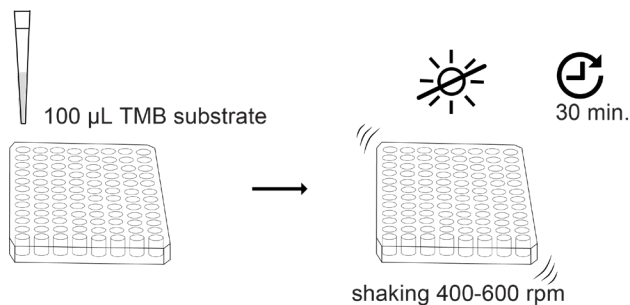
12. Add 100 µL of stop solution to all wells. Remove air bubbles with a pipette tip. Proceed to step 13 within 30 minutes.

13. Read the absorbance at 450 nm in a microtiter plate reader.



100 µL stop solution

Within 30 min.

Read absorbance at 450 nm
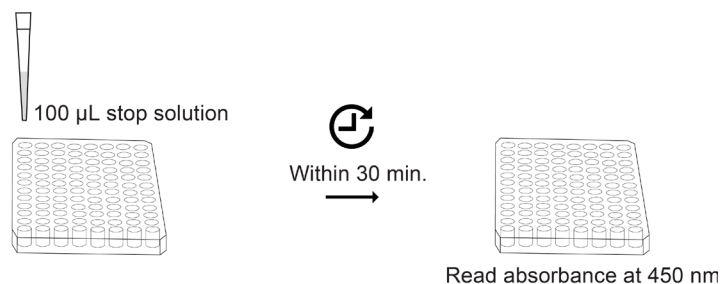
## QUALITY CONTROL

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 anti-GM1 Antibodies 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 in serum samples. The anti-GM1 Antibodies ELISA is standardized against an internally established reference material. Calibrator values are assigned according to a value transfer protocol (ref. 1), 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.

$$\text{\% Ratio}: \frac{\text{absorbance of samples and controls}}{\text{absorbance of calibrator}} \times 100$$

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

*Note: Results presented in table 3 are examples and are provided for demonstration purposes only.*

## LIMITATIONS

- This test has not been validated for plasmapheresis.
- Intravenous immunoglobulins (IVIg) may affect test results.

## PERFORMANCE CHARACTERISTICS

**Within-laboratory precision: 6.8 – 12.9% 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 serum samples were tested. The results are summarized in table 4.

**Reproducibility: 7.7 – 16.1% 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 serum samples were tested. The results are summarized in table 5.

**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 6.

**High dose hook effect**

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

## 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

## Examples of microtiter plate set-ups measuring IgG and IgM-isotypes of 4 samples (figure 1a) or 36 samples (figure 1b)



Figure 1a



Figure 1b

## Example of Results

| Enzyme label | Absorbance (OD$_{450}$) | | Ratio [%] | |
|---|---|---|---|---|
| **B-GCO-ELG/ B-GCO-ELM** | **IgG** | **IgM** | **IgG** | **IgM** |
| Calibrator | 2.397 | 2.269 | | |
| | 2.449 | 2.343 | | |
| **Calibrator Avg.** | **2.423** | **2.306** | **100.0** | **100.0** |
| Medium Control | 1.612 | 1.583 | 67 | 69 |
| | 1.543 | 1.658 | 64 | 72 |
| **Medium Control Avg.** | **1.577** | **1.620** | **65** | **70** |
| Low Control | 0.694 | 0.805 | 29 | 35 |
| | 0.714 | 0.817 | 29 | 35 |
| **Low Control Avg.** | **0.704** | **0.811** | **29** | **35** |
| Negative Control | 0.055 | 0.091 | 2 | 4 |
| | 0.064 | 0.090 | 3 | 4 |
| **Negative Control Avg.** | **0.059** | **0.090** | **2** | **4** |
| Sample 1 | 0.783 | 2.197 | 32 | 95 |
| | 0.802 | 1.988 | 33 | 86 |
| **GM1** | **0.793** | **2,095** | **33** | **91** |

Table 3

## Within-laboratory precision

| Sample Description | | Within-Laboratory Precision | | | |
|---|---|---|---|---|---|
| | **Enzyme Label (Isotype)** | **N** | **Mean [%Ratio]** | **SD [%Ratio]** | **CV [%]** |
| anti-GM1 Ab | IgM | 80 | 48 | 3.5 | 7.2 |
| | | 80 | 91 | 6.2 | 6.8 |
| | IgG | 80 | 40 | 5.1 | 12.9 |
| | | 80 | 106 | 13.1 | 12.4 |

Table 4

## Reproducibility

| Sample Description | | Reproducibility | | | |
|---|---|---|---|---|---|
| | **Enzyme Label (Isotype)** | **N** | **Mean [%Ratio]** | **SD [%Ratio]** | **CV [%]** |
| anti-GM1 Ab | IgM | 75 | 51 | 4.9 | 9.7 |
| | | 75 | 94 | 7.2 | 7.7 |
| | IgG | 75 | 39 | 5.6 | 14.5 |
| | | 75 | 106 | 17.1 | 16.1 |

Table 5

## LoD and LoB

| | Enzyme Label (Isotype) | LoB [% Ratio] | LoD [% Ratio] |
|---|---|---|---|
| anti-GM1 Ab | IgM | 5 | 21 |
| | IgG | 6 | 15 |

Table 6

## anti-GM1 Antibodies ELISA

**Precoated microtiter plate**

↓ ⟲     *wash 2 x with ≥300 µL (cold)! wash buffer*

**100 µL calibrator, controls or**
**serum samples (1:50)**

↓ ⟲     *incubate 2 hours (±5 min) at 2-8 °C*

   ⟲     *wash 3 x with ≥300 µL (cold)! wash buffer*

**add 100 µL enzyme label(s)**

↓ ⟲     *incubate 2 hours (±5 min) at 2-8 °C*

   ⟲     *wash 3 x with ≥300 µL (cold)! wash buffer*

**add 100 µL TMB substrate (ambient temperature)!**

↓ ⟲     *incubate 30 min (±2 min) at 18-28 °C*
                 *on a plate shaker ~400-600 rpm*

**add 100 µL stop solution (ambient temperature)!**

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

### TIME TO RESULT: 4.5 HOURS

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### REFERENCES

1. Blirup-Jensen, S., Johnson, A. M. & Larsen, M. Protein standardization V: Value transfer. A practical protocol for the assignment of serum protein values from a Reference Material to a Target Material. *Clin. Chem. Lab. Med.* **46,** 1470–1479 (2008)

## CHANGELOG

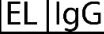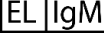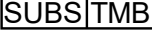| Date | Version | Change |
|------|---------|--------|
| 2023-08-17 | A2 | Change to the *Intended use* and product name<br>Rewording of the *Principle of the assay*<br>New in use stabilities of reagents<br>Update to chapter *Warnings and Precautions*<br>Revision of chapters *Specimen collection and storage, Assay Procedure, Standardization and metrological traceability*<br>Rewording of chapter *Quality Control*<br>Update to chapter *Limitations*<br>Revision of chapters *Performance characteristics* and *Interfering substances*<br>Revision of chapters *References* and *Symbols* |

## 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 |
| SUBS TMB | TMB Substrate |
| SOLN STOP | Stop Solution |