BÜHLMANN GanglioCombi® *Light* ELISA

with enzyme labels IgG/IgM Mix, IgG and IgM

Detection of anti-ganglioside antibodies by ELISA

(GM1, GD1b, and GQ1b)

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

EK-GCL-S-U 96 tests

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Version A1

Manufacturer
BÜHLMANN Laboratories AG
Baselstrasse 55
4124 Schönenbuch, Switzerland
Tel.: +41 61 487 12 12
Fax: +41 61 487 12 34
info@buhlmannlabs.ch

ENGLISH

INTENDED USE

The BÜHLMANN GanglioCombi® *Light* ELISA is an assay for the semi-quantitative determination of IgG and/or IgM antibodies against selected neural antigens/epitopes in serum samples.
For research use only. Not intended for use in diagnostic procedures.

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.
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® *Light* ELISA allows the selective measurement of ganglioside antibodies in serum. The microtiter plate is coated with gangliosides: GM1, GD1b and GQ1b.
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/IgM, anti-IgG, anti-IgM) conjugated to horseradish peroxidase (HRP) detect the anti-ganglioside bound to the immobilized gangliosides 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.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Microtiter Plate precoated with gangliosides	12 x 8 well strip with frame	B-GCL-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 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	1 vial 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	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 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

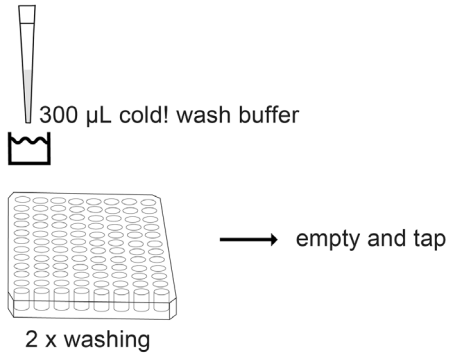
Note: Adjust TMB substrate solution to room temperature (18-28 °C).

1. Dilute samples 1:50 with incubation buffer. Use e.g. 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 (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 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 option2).)

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-E1 (refer to figure 1A or 1B).

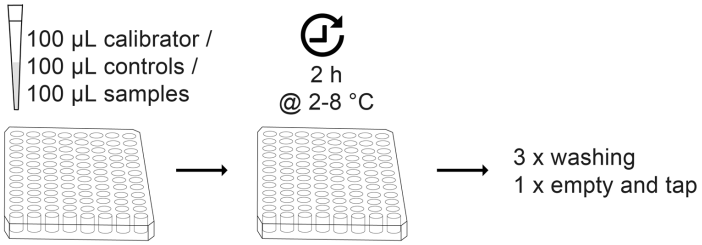
4d. Pipet 100 µL of diluted sample 2 into wells F1-H1 (refer to figure 1A or 1B)

4e. Pipet 100 µL of diluted samples 3-24 (for option 1) or 3-12 (for option2) into subsequent wells (refer to figure 1A or 1B).

Note for option2: 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 washing buffer completely.



For option 1: Detection of mix-isotype

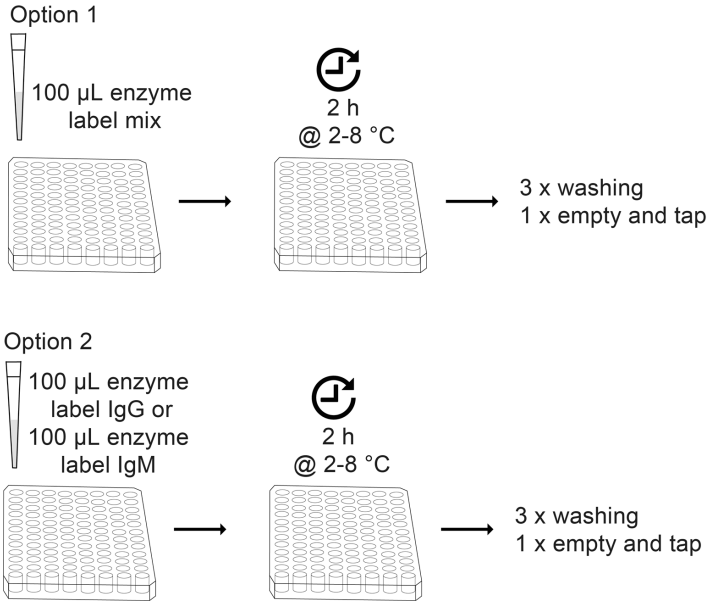
7. Add 100 µL of mix to the wells.

For option 2: Detection of IgG and IgM isotypes

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

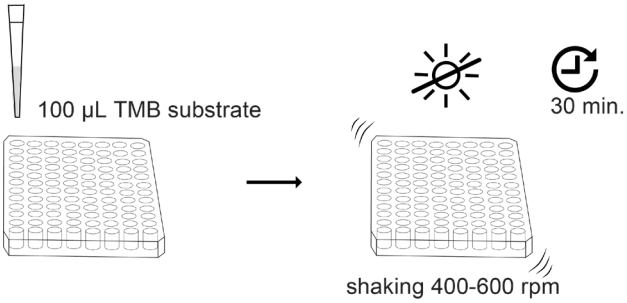
8. Cover the plate with a plate sealer and incubate for 2 hours (±5 min) at 2-8 °C (do not shake the plate).

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 the wells and tap the plate firmly onto blotting paper.



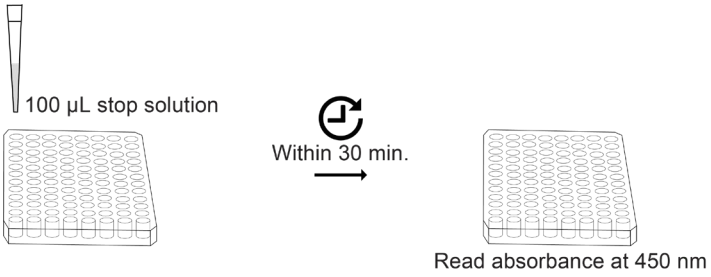
10. Add 100 µL of TMB substrate solution (equilibrated to room temperature) to each well.

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® *Light* 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 BÜHLMANN GanglioCombi® *Light* 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.

Mix isotypes

$$\%\ \text{Ratio:} \quad \frac{\text{absorbance of samples or controls}}{\text{absorbance of calibrator}} \times 200$$

IgG and IgM isotypes

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

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

Note: Results presented in tables 3 and 4 are examples and are provided for demonstration purposes only.

LIMITATIONS

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

PERFORMANCE CHARACTERISTICS

Within-laboratory precision: 5.7 – 13.2% 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 5.

Reproducibility: 7.7 – 19.1% 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 6.

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 7.

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

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

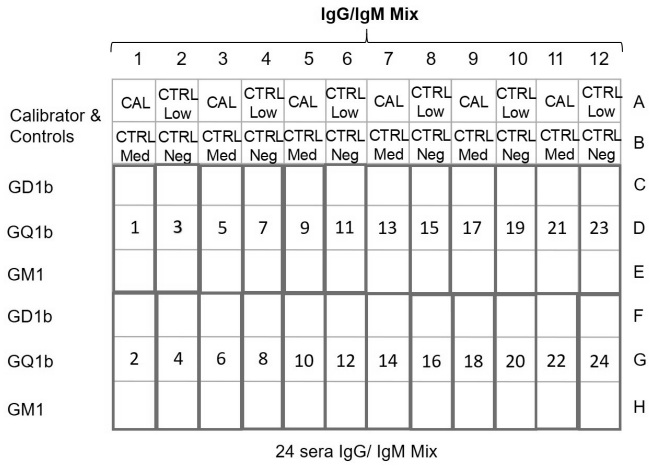


Figure 1A: ≤ 24 sera / kit (1 MP / kit)

Microtiter plate set-up: IgG & IgM labels

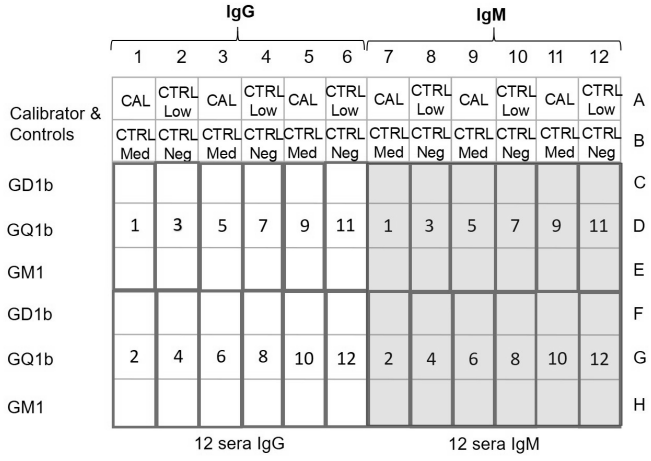


Figure 1B: 2 profiles / serum, ≤ 12 sera / kit (1 MP/kit)

Example of results

A IgG/IgM-Mix label

B-GCO-ELGM	Absorbance (OD450)	Ratio [%]
Calibrator	1.415	
	1.445	
Calibrator Avg.	1.430	200
Medium Control	0.498	69
	0.482	67
Medium Control Avg.	0.490	68
Low Control	0.195	27
	0.191	26
Low Control Avg.	0.193	27
Negative Control	0.090	12
	0.100	14
Negative Control Avg.	0.095	13
Sample 1 GM1	0.544	76
Sample 1 GD1b	0.745	104
Sample 1 GQ1b	0.090	13

Table 3

B IgG & IgM labels

Enzyme labels	Absorbance (OD450)		Ratio [%]	
B-GCO-ELG/ B-GCO-ELM	IgG	IgM	IgG	IgM
Calibrator	1.789	2.576		
	1.833	2.527		
Calibrator Avg.	1.836	2.551	100	100
Medium Control	1.267	1.743	69	68
	1.237	1.764	67	69
Medium Control Avg.	1.252	1.753	68	69
Low Control	0.567	0.938	30	37
	0.584	0.942	32	37
Low Control Avg.	0.571	0.940	31	37
Negative Control	0.061	0.098	3	4
	0.051	0.095	3	4
Negative Control. Avg.	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

TABLES AND FIGURES

Within-laboratory precision

Sample Description		Within-Laboratory Precision			
Analyte	**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
anti-GQ1b Ab	IgM	80	45	2.6	5.7
		80	85	6.7	7.8
	IgG	80	43	5.7	13.2
		80	80	6.9	8.6

Table 5

Reproducibility

Sample Description		Reproducibility			
Analyte	**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
anti-GQ1b Ab	IgM	75	48	3.9	8.2
		75	92	9.9	10.7
	IgG	75	42	8.1	19.1
		75	78	12.0	15.4

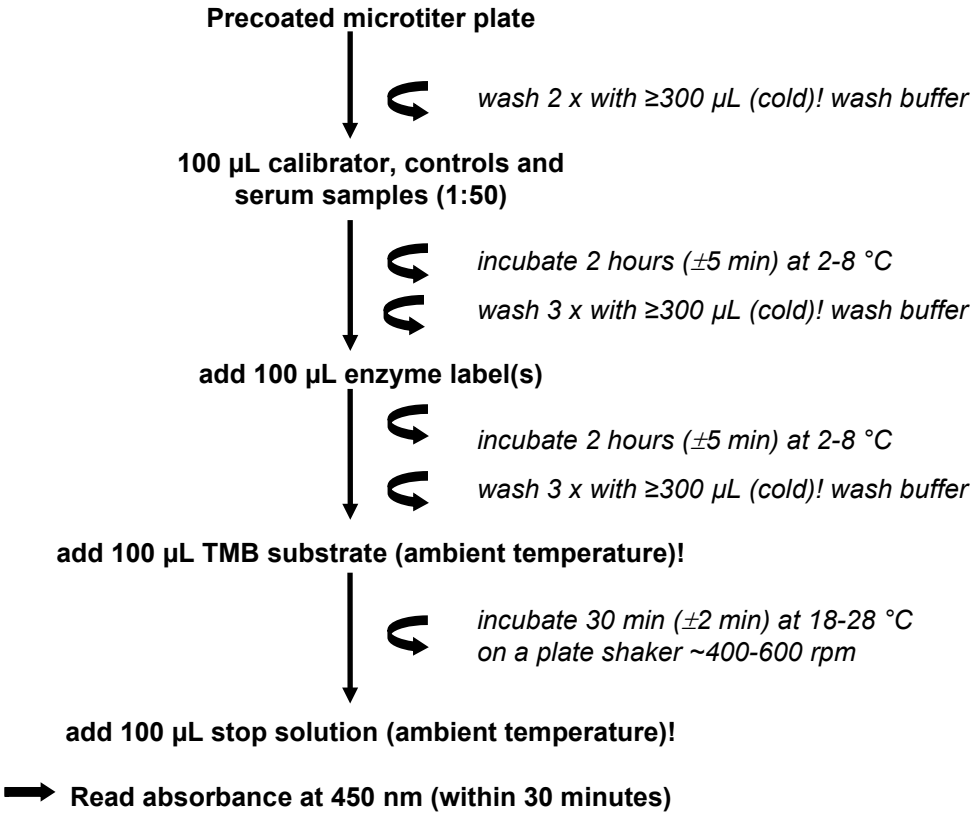
Table 6

LoD and LoB

Analyte	LoB [% Ratio]	LoD [% Ratio]
Anti-GM1- IgM Ab	5	21
Anti-GM1- IgG Ab	6	15
Anti-GQ1b IgM Ab	3	17
Anti-GQ1b IgG Ab	8	18

Table 7

BÜHLMANN GanglioCombi® *Light* ELISA



TIME TO RESULT: 4.5 HOURS

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	A1	Change to the *Intended use* and product name Rewording of the *Principle of the assay* New in use stabilities of reagents Update to chapter *Warnings and Precautions* Revision of chapters *Specimen collection and storage, Assay Procedure, Standardization and metrological traceability (incl. reference)* Rewording of chapter *Quality Control* Update to chapter *Limitations* Revision of chapters *Performance characteristics* and *Interfering substances* Introduction of chapter *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
EL MIX	Enzyme Label IgG/IgM Mix
SUBS TMB	TMB Substrate
SOLN STOP	Stop Solution