# **BÜHLMANN**

# Anti-GM1 Antibodies ELISA

### with enzyme labels IgG and IgM

Detection of anti-ganglioside M1 antibodies by ELISA

For In Vitro Diagnostic Use

EK-GM1-GM 96 tests

Release date: 2023-08-17 Version A2

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#### **INTENDED USE**

The anti-GM1 Antibodies ELISA is an *in vitro* diagnostic assay for the semi-quantitative determination of IgG and/or IgM antibodies against the neural antigen/epitope GM1 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.

#### 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.

Patient sera, controls and calibrator are added to the wells of the microtiter plate. After 2 hours of incubation at  $2 - 8^{\circ}$ 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 and can be assigned to titer categories (negative, grey zone, positive).

#### **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
<b>Calibrator</b> lyophilized with preservatives	1 vial	B-GCO-CA	Add 1.5 mL of Incubation Buffer
Control Negative, Low and Medium <sup>1</sup> 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

Reagents	Quantity	Code	Reconstitution
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

<sup>1</sup> 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 $^\circ\text{C}.$ 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					
Incubation Buffer					
Enzyme Labels	Store for up to 6 months at 2-8 °C.				
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 2methyl-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. <u>Steps 3-9:</u> 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

- <u>Wash steps 3, 6 and 9</u> 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

• <u>Step 11:</u> 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  $\mu$ L 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.

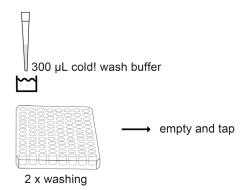
#### ASSAY PROCEDURE

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

- 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 <u>at 2-8 °C for 30</u> <u>minutes</u> 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 <u>without delay</u>. Store refrigerated.

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

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.

#### Detection of IgG-Isotype:

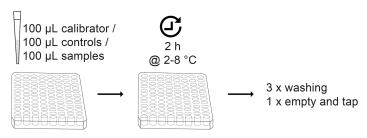
- 4a. Pipet 100  $\mu L$  of calibrator into the well A1 (refer to figure 1a).
- 4b.Pipet 100  $\mu$ L of medium control into well B1, of low control into well C1 and of negative control into well D1 (refer to figure 1a).
- 4c.Pipet 100 μL of diluted sample 1 into the well E1 (refer to figure 1a).
- 4d. Pipet 100  $\mu$ L of diluted sample 2 into the wells F1 (refer to figure 1a).
- 4e.Pipet 100  $\mu$ L of diluted samples x-y into the subsequent wells (refer to figure 1a).

#### **Detection of IgM-Isotype**

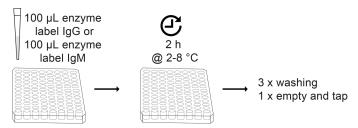
4f. Repeat step 4a-4e using the subsequent wells.

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.

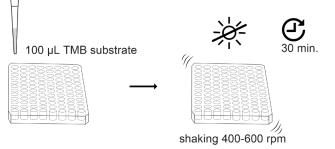
- 5. Cover the plate with a plate sealer and incubate for 2 hours (±5 min) at 2-8 °C (do not shake the plate).
- Remove the plate sealer. Empty the wells and wash three times using at least 300 μL of cold! (2-8 °C) wash buffer per well. Empty the wells and tap the plate firmly onto blotting paper in order to remove wash buffer completely.



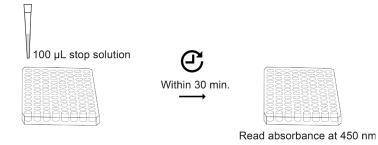
- Add 100 µL of either <u>enzyme label IgG</u> or <u>IgM</u> to the respective wells (refer to figure 1).
- 8. Cover the plate with a plate sealer and incubate for 2 hours (± 5 min) at 2-8 °C (do not shake the plate).
- Remove the plate sealer. Empty the wells and wash three times using at least 300 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.
- 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 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.

absorbance of samples and controls

% Ratio : \_\_\_\_\_\_ x 100

absorbance of calibrator

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

Note: Results presented in table 5 are examples and are provided for demonstration purposes only.

#### LIMITATIONS

- 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. 2).
- 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 anti-GM1 Antibodies ELISA was established according to CLSI C28-A3 with 120 serum samples from self-declared healthy individuals. Distribution frequency of anti-GM1-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 6. The cut-off value for positivity is 50% Ratio.

#### **RESULT INTERPRETATION**

Ganglioside	IgG or IgM Values (% Ratio)			
	<30	30-50	>50	
GM1	Negative	Retest at a later time point	Positive	

Table 3

Test results should be interpreted in conjunction with information available from the clinical assessment of the patient and other diagnostic procedures.

#### 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 patient serum samples were tested. The results are summarized in table 7.

#### 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 patient serum samples were tested. The results are summarized in table 8.

### 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 9.

#### 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 10) and from patients with other neurological disorders (table 11).

#### **CLINICAL PERFORMANCE**

The clinical performance was assessed by descriptive analysis of peer-reviewed scientific literature. Four (4) studies addressed the clinical performance of the Anti-GM1 Antibodies ELISA in the diagnosis of autoimmune peripheral neuropathies (ref. 3-6). Results of analysis and study details are provided in table 4 and table 12, respectively.

N peripheral neuropathy	148 (102 pediatric GBS, 14 CIDP, 32 GBS)
N controls	341 (70 DC, 142 NC, 129 HC)
Sensitivity, mean (95% CI)	48.7 % (27.9 – 69.5 %)
Specificity, mean (95% CI)	77.8 % (60.9 – 94.7 %)
	Table 4

GBS, Guillain-Barré-Syndrome; CIDP, Chronic Inflammatory Demyelinating Polyneuropathy; DC, Non-Neurological Disease Control; NC, Neurological Control; HC, Healthy Control; CI, confidence interval

#### 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  $\geq \pm 20\%$  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  $\mu$ g/mL), ibuprofen (0.22 mg/mL), chlorambucil (1.96  $\mu$ g/mL), prednisone (99 ng/mL), prednisolone (1.2  $\mu$ g/mL), rheumatoid factor (2340 IU/mL), hemoglobin (10 mg/mL), hemolysate (10 mg/mL), triglyceride (15 mg/mL), conjugated bilirubin (20  $\mu$ g/mL), unconjugated bilirubin (150  $\mu$ g/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)

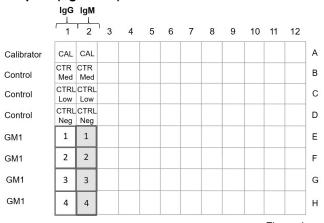


Figure 1a

	lgG				lgM								
	1	2	3	4	5	6	7	8	9	10	11	12	
Calibrator &	CAL	CTRL Low	CAL	CTRL Low	CAL	CTRL Low	CAL	CTRL Low	CAL	CTRL Low	CAL	CTRL Low	A
Controls	CTRL Med	CTRL Neg	CTRL Med	CTRL Neg		CTRL Neg	CTRL Med	CTRL Neg	CTRL Med	CTRL Neg	CTRL Med	CTRL Neg	В
GM1	1	7	13	19	25	31	1	7	13	19	25	31	С
GM1	2	8	14	20	26	32	2	8	14	20	26	32	D
GM1	3	9	15	21	27	33	3	9	15	21	27	33	Е
GM1	4	10	16	22	28	34	4	10	16	22	28	34	F
GM1	5	11	17	23	29	35	5	11	17	23	29	35	G
GM1	6	12	18	24	30	36	6	12	18	24	30	36	н
											Figu	ire 1	b

#### Example of results

Enzyme label		Absorbance (OD <sub>450</sub> )		Ratio [%]
B-GCO-ELG/	lgG	lgM	lgG	lgM
B-GCO-ELM				
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
	•	1		Table 5

#### **Reference interval**

	Enzyme Label	% normal blood donors in categories			Reference limit
	(Isotype)	<30 %Ratio	30 - 50 %Ratio	>50 %Ratio	(90% CI)
anti-GM1	lgG	99.2	0.8	0.0	16 (13.0 – 29.8)
Ab	IgM	95.8	3.3	0.8	24 (14.3 – 40.3)
					Table 6

#### Within-laboratory precision

Sample Description			Within-Laboratory Precision			
	Enzyme Label (Isotype)	Expected Category [%Ratio]	N	Mean [%Ratio]	SD [%Ratio]	сv [%]
	la M	30-50	80	48	3.5	7.2
anti-GM1	lgM	>50	80	91	6.2	6.8
Ab	lgG	30-50	80	40	5.1	12.9
		>50	80	106	13.1	12.4
Table 7						

#### Reproducibility

Sample Description				Reproducibility			
	Enzyme Label (Isotype)	Expected Category [%Ratio]	N	Mean [%Ratio]	SD [%Ratio]	сv [%]	
	IgM	30-50	75	51	4.9	9.7	
anti-GM1		>50	75	94	7.2	7.7	
Ab	In C	30-50	75	39	5.6	14.5	
	lgG	>50	75	106	17.1	16.1	
					1	Table 8	

#### LoD and LoB

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

Table 9

#### **Cross-reactivity**

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

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 1

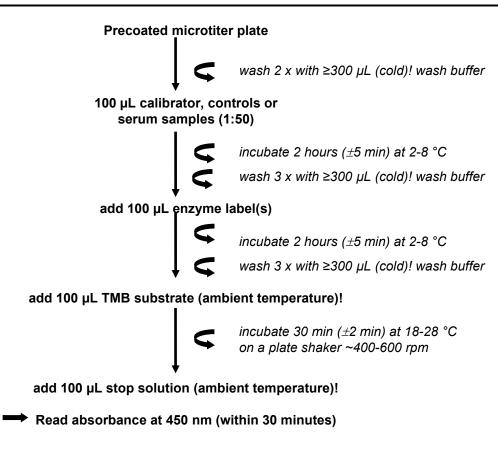
#### TABLES AND FIGURES

#### **Clinical performance**

Study	Positive controls (Cases)	Negative controls	Epi- tope	Sensi- tivity	Speci- ficity
Hashemilar et al., 2014	Pediatric GBS (n = 45)	DC (n = 35)	GM1	0.51	0.89
Sharma et al., 2011	Pediatric GBS	NC (n = 42)	GM1	0.82	0.33
	(n = 57)	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	lmen et (n = 19, 14)	NC (n = 100)	GM1	0.30	0.93
		HC (n = 110)			0.95
					Table 12

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





**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).

2. Bourque, P. R. et al. Autoimmune peripheral neuropathies. Clinica Chimica Acta 449, 37-42 (2015).

3. Hashemilar, M. et al. Evaluating the status of antiganglioside antibodies in children with Guillain-Barré syndrome. Neuroimmunomodulation 21, 64–68 (2013).

4. Sharma, M. B. et al. The presence of Mycoplasma pneumoniae infection and GM1 ganglioside antibodies in Guillain-Barré syndrome. J. Infect. Dev. Ctries. 5, 459–464 (2011).

5. Uetz-von Allmen, E. et al. Antiganglioside GM1 antibodies and their complement activating capacity in central and peripheral nervous system disorders and in controls. Eur. Neurol. 39, 103–110 (1998).

6. Khandelwal, D. et al. IgM anti-GM1 antibody titers in patients with monomelic amyotrophy. Neurol. India 54, 399–401 (2006).

#### CHANGELOG

Date	Version	Change
2023-08-17	A2	Change to the <i>Intended use</i> and product name Rewording of the <i>Principle of the assay</i> with titer categories negative, grey zone, positive New in use stabilities of reagents Update to chapter <i>Warnings and Precautions</i> Revision of chapters <i>Specimen collection and storage, Assay Procedure,</i> <i>Standardization and metrological traceability</i> Rewording of chapter <i>Quality Control</i> Update to chapter <i>Limitations</i> Introduction of chapter <i>Result Interpretation</i> Revision of chapters <i>Reference Intervals and cut-off, Performance characteristics</i> and <i>Interfering substances</i> Introduction of chapter <i>Clinical Performance</i> Revision of chapters <i>References</i> and <i>Symbols</i> 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)
BUFINC	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
SOLNSTOP	Stop Solution
	<b>EN</b> : electronic instruction for use available in different languages at:/ <b>BG</b> : електронни инструкции за употреба на различни езици на адрес:/ <b>CS</b> : elektronický návod k použití dostupný v různých jazycích na adrese:/ <b>DA</b> : elektronisk brugsanvisning på forskellige sprog på:/ <b>DE</b> : elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ <b>EL</b> : ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ <b>ES</b> : instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ <b>ET</b> : elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ <b>FR</b> : un mode d'emploi électronique disponible en différentes langues à l'adresse:/ <b>HU</b> : különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ <b>IT</b> : istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ <b>LT</b> : elektronics naudojimo instrukcijos įvairiomis kalbomis:/ <b>LV</b> : dažādās valodās pieejama elektroniska lietošanas instrukcija:/ <b>NO</b> : elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ <b>PL</b> : elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ <b>PT</b> : instrução electrónica para utilização disponível em diferentes línguas em:/ <b>RO</b> : instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ <b>SK</b> : elektronický návod na použitie dostupný v rôznych jazykoch na:/ <b>SL</b> : elektronska navodila za uporabo so na voljo v različnih jezikih na:/ <b>SR</b> : elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:/ <b>SV</b> : elektronisk bruksanvisning på olika språk på följande adress:

# **CE**<sub>0123</sub>