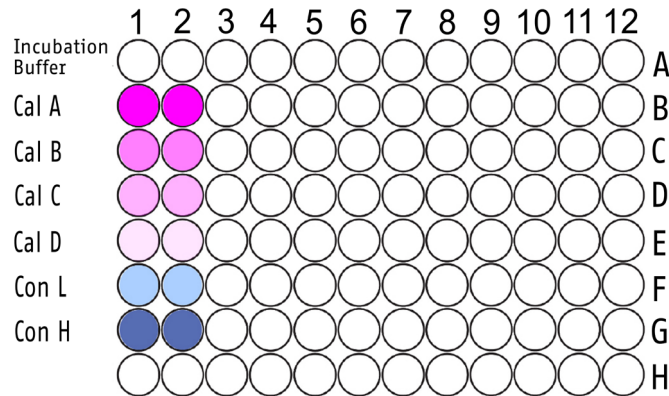


anti-MAG Antibodies ELISA

Pre-Analytics

Specimen	Serum
Specimen collection	Use conventional plain tubes to collect blood. Process to serum according to manufacturer's instruction.
Storage of Serum	<ul style="list-style-type: none"> • Prepare aliquots • Freeze/thaw cycles not recommended • 2-8°C (up to 16 days) • -20°C (up to 12 months)

Microtiter Plate Layout



- Uniformly coated with human MAG
- Break-away strip convenience

Number of Analysis

Number of test runs	Number of analysis
1	at least 41
2	at least 34
3	at least 27

Reagent Preparation

Reagent	Temperature °C
Wash buffer*	2-8
Incubation Buffer*	2-8
Enzyme conjugate*	2-8
TMB substrate	18-28
Stop solution	18-28

* New Formulation: Contain eco-friendly Tween 20.

Sample Preparation

- Dilute samples 1:1000 with incubation buffer and vortex gently.
- Leave diluted samples, reconstituted calibrators and controls at 2-8°C for 30 minutes prior pipetting.

Procedure

Preparation: Sera, Calibrators, Controls

- ↓ Dilute Sera: 1:1'000 (incubation buffer)
- ↻ Reconstitute Calibrators and Controls

Precoated Microtiter Plate

- ↓ ↻ wash 4x (≥ 300 µL wash buffer)

Sera, Calibrators, Controls (100 µL, each)

- ↓ ↻ *incubate* (2 hr ± 5 min)

- ↓ ↻ wash 4x (≥ 300 µL wash buffer)

Enzyme Label (100 µLs)

- ↓ ↻ *incubate* (2 hr ± 5 min)

- ↓ ↻ wash 4x (≥ 300 µL wash buffer)

TMB Substrate (100 µL)

- ↓ ↻ *incubate* (30 min ± 2 min) on a plate shaker: 400 – 600 rpm

Stop Solution (100 µL)

- ↓ ↻ within 30 min

Absorbance (450 nm)

cold
(2 - 8°C)

ambient
(18 - 28°C)

Literature References

Supporting clinical performance and cut-off value

Kuijff M. et al., Neurology, 2009
 Stork A.J.C. et al., J Neuroimmunol, 2014 and 2016
 Campagnolo M. et al., J Neuroimmunol, 2015
 Vallat J.M. et al., Neurology, 2021
 Steck A. J et al., J Neuroimmunol, 2021

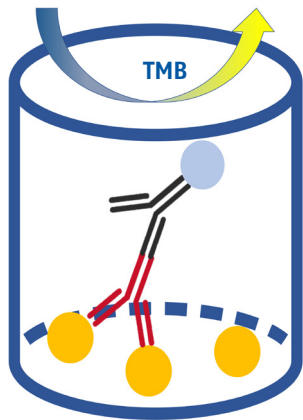
This document is for information purpose only, before performing the assay please carefully refer/read the respective IFU available (<https://www.buhlmannlabs.ch/support/downloads/eifus/>).

anti-MAG Antibodies ELISA

Methodology

Method	ELISA For laboratory use only
Analyte	anti-MAG Antibodies (IgM)
Test	Semi-quantitative ELISA
Suggested Use	<ul style="list-style-type: none"> • Stand-alone-test or • Verification of samples displaying (either IgM or IgG/IgM) HNK-1 positivity of HNK-1 (IgM or IgG/IgM Mix) as determined with BÜHLMANN GanglioCombi® MAG ELISA (EK-GCM)

Principle of the Assay



color reaction measured at 450nm

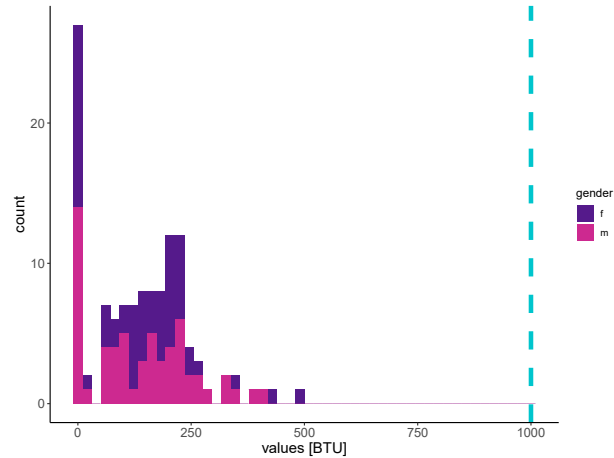
2nd anti IgM-antibody

Analyte: anti-MAG antibodies

Antigen: purified human MAG precoated on microtiter Plate

Reference Interval

Determined from N=239 Controls, Apparently Normal Healthy Donors (n=141) and samples from Donors Various Autoimmune Conditions (n=98). The reference interval is < 1000 BTU.



Clinical Performance

The clinical performance was assessed by meta-analysis of peer-reviewed scientific literature. Various studies addressed the clinical performance of the anti-MAG Antibodies ELISA in the diagnosis of IgM monoclonal gammopathy-associated neuropathies. The studies confirmed a cut-off of 1000 BTU.

Neuropathies of various etiology	344
Number of positive samples	477
Sensitivity (95% CI)	58.9% (47.2-69.6%)
Specificity (95% CI)	98.2% (89.7-99.7%)
ROC AUC	0.75
<small>(Area under receiver operating characteristics curve)</small>	

Results

- The measurement of absorbance is proportional to the titer of anti-MAG antibodies in a given sample.
- Standard Curve (curve function): 4-parameter-logistic (4-PL).
- Titers: expressed as BÜHLMANN Titer Units (BTU).
- Lot-specific Control values (low and medium) are provided on the QC-data sheet (included in each kit).

BÜHLMANN Titer Units (BTU)

BTUs are antibody-titer levels calculated in function of dilutions at which a reference pool generates a signal corresponding to the cut-off value, thus excluding inter-assay (OD) bias.

Suggested Handling of Titers

(Vallat J.-M et al., Neurology, 2021)

Titer levels [%Ratio]	Interpretation [titer]
< 1000	negative
1000 - 10000	low
10000 - 70000	mid
≥ 70000	high

Ordering code:
EK-MAG 96 tests (IgM)

CE 0123

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