

BÜHLMANN GanglioCombi® MAG 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

- Prepare aliquots
- Freeze/thaw cycles not recommended
- 2-8°C (up to 16 days)
- -20°C (up to 12 months)

Number of Analysis

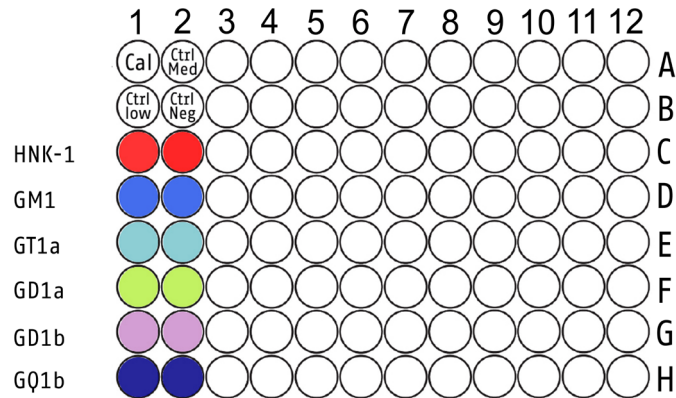
- GanglioCombi MAG ELISA allows for full flexibility regarding:
 - number of samples to be pipetted
 - use and arrangement of enzyme conjugates on microtiter plates
- A sample is investigated for 6 different antibodies with IgG and IgM and/or with IgG/IgM mix conjugate.
- Each kit contains 2 microtiter plates.

Enzyme Conjugate	Number of Analysis per Kit	Number of Analysis per individual Sample
IgG & IgM	12 + 12	2 x 6 (2 conjugates / 6 antigens)
IgG/IgM Mix	24	1 x 6 (1 mix conjugate / 6 antigens)

Literature References

Kuijff M et al., Journal of the Neurological Sciences, 2005
 Spatola M et al., Neurology, 2016
 Franciotta D et al., Clin Chem Lab Med, 2018
 Delmont E et al., Journal of Neurology, 2019

Microtiter Plate Layout



- Coated in rows with HNK-1 (C1 - C12) and individual gangliosides from D1 to H12
- Break-away strip convenience

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

Sample Preparation

1. Dilute samples 1:50 with incubation buffer and vortex gently.
2. Leave diluted samples, reconstituted calibrators and controls at 2-8°C for 30 minutes prior pipetting (for targeted investigation).

ELISA Procedure

Precoated Microtiter Plate

↓ ↻ wash 2x (≥ 300 µL wash buffer)

Calibrators, Controls, Sera (100 µL; dilution of sera 1:50)

↓ ↻ incubate (2 hr ± 5 min)

↓ ↻ wash 3x (≥ 300 µL wash buffer)

Enzyme Label (100 µL)

↓ ↻ incubate (2 hr ± 5 min)

↓ ↻ wash 3x (≥ 300 µL wash buffer)

TMB Substrate (100 µL)

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

Stop Solution (100 µL) ≤ 30 min

↓ Absorbance (450 nm)

cold
(2 - 8°C)

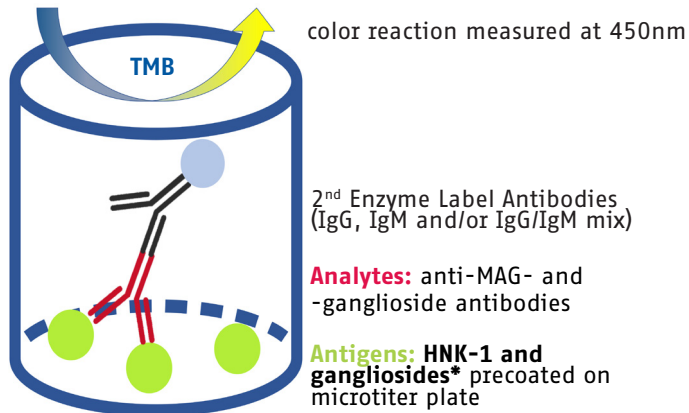
ambient
(18 - 28°C)

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Methodology

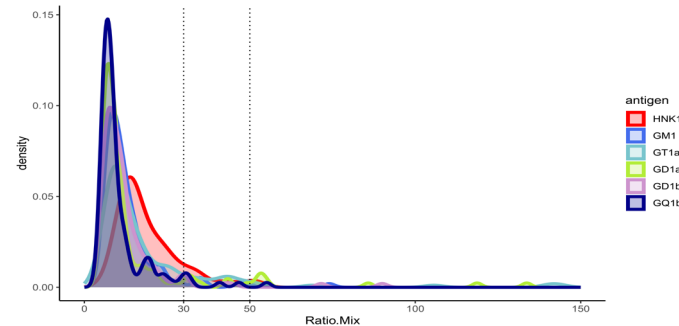
Method	ELISA For laboratory use only
Analyte	anti-MAG* and -ganglioside** antibodies *Antigen: HNK-1 chemically synthesized sulphated disaccharide **Gangliosides: GM1, GD1a, GD1b, GT1a, GQ1b
Test	multi-parametric, semi-quantitative
Suggested Use	Targeted investigation of most relevant anti-MAG and/or -ganglioside antibodies of IgG, IgM and/or IgG/IGM Mix. anti-HNK-1 antibodies >50% Ratio may be confirmed with anti-MAG Antibodies ELISA (ordering code: EK-MAG)

Principle of the Assay



*We use double-quality control-checked gangliosides for the coating of the microtitre plates

Reference Interval



Healthy Individuals: N = 120 (m = 60; f = 60)
Negative: 90.8%; Grey zone: 6.3%; Positive: 2.9%

¹ Autoimmune diseases (N = 50): Vasculitis and ANCA positive denoted: 3 and 10; SLE: 5; RA: 9 Sjögren Syndrome: 6; Others (ANA positive denoted): 3; Autoimmune Thyroiditis: 5; MCTD: 1; Autoimmune peripheral neuropathies (GM1 and GQ1b pos): 1; MG: 7

² Other neurological diseases (N = 34): Alcoholic: 1; diabetic: 5; ALS: 15; Sarcoidosis: 4; WM: 4; Chagas Disease: 5

Performance Data

Crossreactivity:	No (incl. AIDs ¹ and ONDs ²)
Reproducibility (%CV):	7.7–19.1% (anti-ganglioside antibodies) 23.5–33.2% (anti-MAG antibodies)
Specificity (95 %CI):	88.0% (72.3–95.3%)
Sensitivity (95 %CI):	68.1% (39.6–87.5%)

Results

- The measurement of absorbance is proportional to the titer of (MAG- and/or -gangliosides) antibodies in a given sample.
- Calibrators and Controls are anti-GM1 antibodies
 - Titers are expressed as %Ratio of anti-GM1 antibodies
 - Calculation:
IgG and IgM: OD Sample/OD Calibrator x 100%;
Mix: OD Sample/OD Calibrator x 200%

Handling of Titer levels

Titer levels [%Ratio]	Interpretation [titer]
< 30	negative
30 - 50	grey zone (equivocal)
> 50	positive

Ordering information:

BÜHLMANN GanglioCombi® MAG ELISA 5 gangliosides, 1xMAG EK-GCM

Variants:

BÜHLMANN GanglioCombi® Light ELISA 3 gangliosides EK-GCL-S

anti-GM1 Antibodies ELISA 1 ganglioside EK-GM1-GM

CE 0123

Parts of the BÜHLMANN GanglioCombi® MAG ELISA kits are patent protected by: CA 2 996 073 and WO 2017/046172 A1



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