BÜHLMANN anti-MAG™ Antibody ELISA

anti-Myelin Associated Glycoprotein Autoantibodies

This product is for research use only
It is not intended for use in diagnostic procedures

EK-MAG-U 96 tests

Revision date: 2015-11-10 US

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ENGLISH

INTENDED USE

The BÜHLMANN anti-MAG™ Antibody ELISA is intended for the quantitative *in vitro* determination of human IgM-autoantibodies directed against Myelin Associated Glycoprotein (MAG) in serum (1, 2). This product is for research use only. It is not intended for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The BÜHLMANN anti-MAG™ Antibody ELISA employs the quantitative enzymatically amplified sandwich-type immunoassay technique. HIGHLY PURIFIED MAG FROM HUMAN BRAIN (2) has been precoated onto a microtiter plate. Calibrators and sera are incubated for two hours in the microtiter wells and any anti-MAG autoantibodies present are bound by the immobilized human MAG. After washing away any unbound substances, horseradish peroxidase (HRP) labeled antibodies against human IgM are added to the wells and incubated for another two hours. After a wash step, the substrate solution containing tetramethylbenzidine (TMB) is added to the wells and incubated for 30 minutes. A blue coloration develops in proportion to the amount of anti-MAG autoantibodies bound in the initial step. The color development is stopped by adding an acidic stop solution (H_2SO_4) which turns the blue solution to yellow. The intensity of the color absorbance is measured in a microtiter plate reader at a wavelength of 450 nm. The absorbance measured is directly proportional to the concentration of anti-human MAG autoantibodies. A set of human anti-MAG autoantibody calibrators is used to plot a standard curve of absorbance versus human anti-MAG autoantibody titer units from which the concentrations of anti-human MAG autoantibodies in the unknowns can be calculated.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Microtiter Plate 96 wells precoated with human MAG	12 x 8-well strips with holder	B-MAG-MP	Ready to use
Plate Sealer	3 pieces		
Wash Buffer Concentrate (10x)	1 bottle 100 ml	B-MAG-WB	Dilute with 900 ml of deionized water
Incubation Buffer with preservatives	1 bottle 100 ml	B-MAG-IB	Ready to use
Calibrators A to D[1] Human serum with preservatives	4 vials	B-MAG-CASET	Add 1 ml of Incubation Buffer
Low and **High Control**[2] Human serum with preservatives	2 vials	B-MAG-CONSET	Add 1 ml of Incubation Buffer
Enzyme Label IgM Anti-human IgM antibody conjugated to HRP in a protein-based buffer with preservatives	1 vial 11 ml	B-MAG-ELM	Ready to use Blue solution
TMB Substrate TMB in Citrate buffer with Hydrogen Peroxide	1 vial 11 ml	B-TMB	Ready to use
Stop Solution 0.25 M Sulfuric acid	1 vial 11 ml	B-STS	Ready to use **Corrosive agent**

Table 1

[1] After reconstitution, Standards A, B, C and D contain 70000, 15000, 3000 and 1000 BÜHLMANN Titer Units (BTU) of anti-MAG antibodies, respectively.

[2] Controls contain lot-specific amounts of anti-MAG antibodies. Refer to the additional control data sheet for exact concentrations.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
Store at 2-8°C. Do not use past kit expiration date printed on the label.	
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 2 months at 2-8°C.
Wash Buffer diluted	Store for up to 2 months at 2-8°C.
Calibrators	Store for up to 2 months at -20°C.
Controls	
Incubation Buffer	Store at 2-8°C until expiration date.
Enzyme Label IgM	
TMB Substrate (protect from light)	
Stop Solution	Store at 18-28°C until expiration date.

Table 2

WARNING AND PRECAUTIONS

Calibrators (B-MAG-CASET) and Controls (B-MAG-CONSET) of this kit contain components of human origin. Each serum donor unit used in the preparation of the kit components was tested by an FDA approved method and found negative for HBV surface antigen, so as for HCV and HIV1/2 antibodies. Although these methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. *Therefore, all human specimens and kit components should be handled as if capable of transmitting infections*. All products containing human source material should be handled in accordance with good laboratory practice using appropriate precautions.

Substrate and Stop Solution: The Substrate Solution (B-TMB) contains Tetramethylbenzidine (TMB), hydrogen peroxide and dimethylformamide. The Stop Solution (B-STS) contains sulfuric acid. Each of those reagents is irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes with disposable tips: 2 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 Wash Buffer Concentrate.
- Microtiter plate washer or squeeze bottle for Wash Buffer.
- Microtiter plate rotator.
- Microtiter plate reader for measurement of absorbance at 450 nm.

SPECIMEN COLLECTION AND STORAGE

The procedure calls for <0.1 ml of blood or <50 µl of serum. Lipemic, hemolytic and icteric samples should not be used in this assay. Lipemic samples can be avoided by asking humans to fast for at least 12 hours prior to the sample being taken. Collect blood into plain tubes, avoid hemolysis, leave to clot for one hour at RT (18-28°C), centrifuge for 15 minutes at approximately 1800 x *g* at RT and collect the serum.

Store serum samples at ≤-20°C. Samples are stable for ≥1 year if stored at ≤-20°C. Avoid repeated freeze-thaw cycles. Frozen samples should be thawed and mixed thoroughly by gentle swirling or inversion prior to use.

ASSAY PROCEDURE

1. Dilute samples 1:1000 with Incubation Buffer (*e.g.* 2 µl of serum + 2 ml of Incubation Buffer). Allow diluted samples to set for one hour at 18-28°C vortex from time to time. Put samples for 10 minutes on ice prior to pipetting in step 4c.
2. Prepare a plate with sufficient strips to test the desired number of calibrators, controls and samples. Remove excess strips from the holder and reseal them in the foil pouch together with the desiccant packs **without delay**. Store refrigerated.

Important: Use refrigerated reagent solutions in steps 3. to 9.

3. Wash the coated wells four times using at least 300 µl of refrigerated Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

4a. Pipet 100 µl of Incubation Buffer (as blank) in duplicate into wells A1+A2.
Pipet 100 µl of Calibrator A in duplicate into wells B1+B2.
Pipet 100 µl of Calibrator B in duplicate into wells C1+C2.
Pipet 100 µl of Calibrator C in duplicate into wells D1+D2.
Pipet 100 µl of Calibrator D in duplicate into wells E1+E2.

4b. Pipet 100 µl of the Low Control in duplicate into wells F1+F2.
Pipet 100 µl of the High Control in duplicate into wells G1+G2.

4c. Pipet 100 µl of each diluted sample in duplicate into the subsequent wells.

5. Cover the plate with a Plate Sealer and incubate for 2 hours (± 5 min) at 2-8°C.
6. Remove and discard Plate Sealer. Empty wells and wash four times using at least 300 µl of **refrigerated** Wash Buffer per well. Empty wells and strike plate firmly onto blotting paper.
7. Add 100 µl of Enzyme Label IgM to all wells.
8. Cover plate with a Plate Sealer and incubate for 2 hours (± 5 min) at 2-8°C.
9. Remove and discard Plate Sealer. Empty wells and wash four times using at least 300 µl of **refrigerated** Wash Buffer per well. Empty wells and strike the plate firmly onto blotting paper.

Important: Allow TMB substrate solution to adapt to 18-28°C.

10. Add 100 µl of TMB Substrate Solution to each well.
11. Cover the plate with a Plate Sealer, place the plate on a plate mixer set at 800-1000 rpm, protect plate from direct light and incubate for 30 minutes (± 5 min) at 18-28°C.
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. If wavelength correction is available, set instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

RESULTS

Standard Curve

- Record the absorbance at 450 nm for each calibrator.
- Average the duplicate values.
- Plot the average absorbance values (vertical y-axis) versus BTU (see next chapter) of the calibrators (horizontal x-axis) using a lin/log graph paper.
- Draw the best fitting curve or calculate the standard curve using 4 parameter curve fit.

Samples and Controls

- Record the absorbance at 450 nm for each sample and control well.
- Average the duplicate values.
- Locate the absorbance values of the samples and controls on the vertical axis, draw a horizontal line intersecting the standard curve and read from the horizontal axis.

NOTE: If the microtiter plate reader is not capable of reading absorbance greater than 2 or greater than the absorbance of the highest calibrator (Calibrator A), a second reading at a wavelength of 490 or 492 nm is recommended (reference filter at 600 or 620 nm if available). In this case, proceed to construct a second standard curve with the absorbance readings of all calibrators at 490 or 492 nm. The concentration of the off-scale samples at 450 nm are then read from the new standard curve as described above. The readings at 490 or 492 nm should not replace the on-scale readings at 450 nm.

Examples of Results and of Standard Curve (see Table 3 and Figure 1)
These results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

STANDARDIZATION

Proposed Cut-off Titer

The mean + 3SD results in a technical cut-off value of 729 BTU. For practical reasons, we recommend to use a CUT-OFF VALUE OF 1000 BTU, corresponding to the lowest calibrator (= Calibrator D) in the standard curve.

QUALITY CONTROL

A thorough understanding of this package insert is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following this package insert.
Since there is no control serum for anti-MAG antibodies commercially available, we recommend to use a positive serum pool for internal quality controls.
All controls must fall within established confidence limits. The confidence limits for the Controls are lot-specific and printed on the additional data sheet.
The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) ELISA reader settings iii) expiration dates of reagents iv) storage and incubation conditions v) TMB Substrate Solution should be colorless vi) purity of water.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (Within-Run): 6.5%. The intra-assay precision was calculated from the results of 20 pairs of values obtained in a single run (*cf.* Table 4).

Inter-Assay Precision (Run-to-Run): 15.4%. The inter-assay precision was calculated from the results of 20 pairs of values obtained in 20 different runs (*cf.* Table 5).

Dilution Linearity/Parallelism: 147%. 7 human serum samples containing high titer of anti-MAG antibodies were diluted with Incubation Buffer 1:1000 to 1:64000, left for one hour at 18-28°C and subsequently assayed according to the assay procedure (cf. Table 6). It is suggested that the relatively high deviation in about 50% of samples is caused by antibody aggregations.

Analytical Sensitivity: 444 BTU. 20 duplicates of incubation buffer were assayed in a single run. Mean and standard deviation were calculated for the absorbance values. The minimal detectable dose of anti-MAG antibodies was calculated to be 444 BTU by adding two standard deviations to the mean absorbance and intersecting this value with the standard curve obtained in this run.

Functional sensitivity: 900 BTU 20 duplicates of low titer anti-MAG autoantibodies were assayed in a single run. Mean, standard deviation (SD) and coefficient of variation (CV) were calculated from the absorbance values. At 900 BTU, the CV was less than 10%.

Table 3: **Examples of results**

	Conc. [BTU]	Absorbance [OD]	Calc. Conc. [BTU]	CV Conc. [%]
Blank 1		0.046		
Blank 2		0.049		
Average		**0.048**		
Calibrator A	70000	2.195	70497	
Calibrator A	70000	2.188	69508	
Average	**70000**	**2.191**	**70000**	**0.2**
Calibrator B	15000	1.272	15313	
Calibrator B	15000	1.245	14693	
Average	**15000**	**1.258**	**15000**	**1.5**
Calibrator C	3000	0.417	3070	
Calibrator C	3000	0.400	2931	
Average	**3000**	**0.408**	**3000**	**2.9**
Calibrator D	1000	0.135	1009	
Calibrator D	1000	0.132	991	
Average	**1000**	**0.134**	**1000**	**1.5**
Control LOW		0.360	2602	
Control LOW		0.376	2731	
Average		**0.368**	**2666**	**3.1**
Control HIGH		1.395	18433	
Control HIGH		1.383	18090	
Average		**1.389**	**18261**	**0.6**
Sample 1		0.001	255	
Sample 1		0.009	297	
Average		**0.005**	**276**	**116.5**
Sample 2		1.092	11599	
Sample 2		0.969	9511	
Average		**1.030**	**10555**	**8.5**

Figure 1: **Example of Standard Curve**

OD_{450}

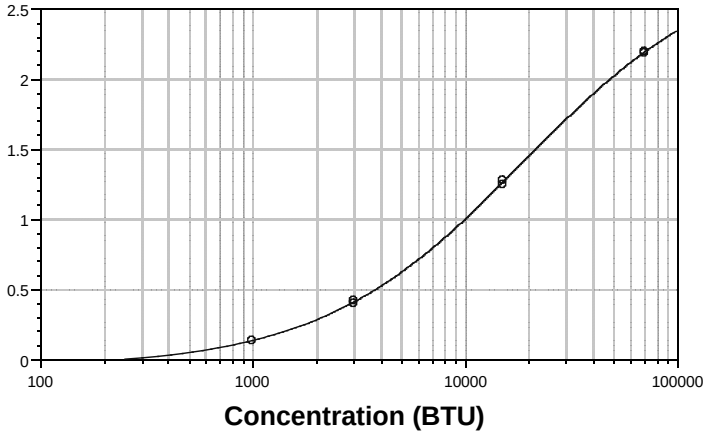

Table 4: **Intra-Assay Precision (Within-Run)**

Sample Type	Mean [BTU]	SD [BTU]	CV [%]
Serum 1 (Low)	3764	176	4.7
Serum 2 (High)	35384	2920	8.3
Mean			6.5

Table 5: **Inter-Assay Precision (Run-to-Run)**

Sample Type	Mean [BTU]	SD [BTU]	CV [%]
Serum 3 (Low)	4208	714	17.0
Serum 4 (High)	17112	2362	13.8
Mean			15.4

Table 6: **Dilution Linearity**

Serum Samples	Range Min – Max	Mean Observed/Expected
5	78 – 96%	86%
6	99 – 119%	107%
7	105 – 151%	123%
8	162 – 229%	206%
9	135 – 231%	196%
10	112 – 292%	233%
11	59 – 98%	76%
Mean		147%

Table description: cf. “Results and Calculations”, “Performance Characteristics

1. Quarles, R.H and Weiss, M.D.: Autoantibodies associated with peripheral neuropathy. Muscle Nerve 22, 800-822 (1999).
2. Burger, D. and Steck, A.J.: Neuropathies associated with anti-myelin antibodies. In: Hohlfeld, R. (Ed.): Immunology of neuromuscular disease 24, 7-32 (1994).
3. Schmit, P. et al.: Evaluation of four different methods for the detection of anti-myelin autoantibodies. Poster presented at the MEDLAB97, 12th IFCC, Europ Congr of Clin Chem, Basel. Abstract C139 (pp 229-230)

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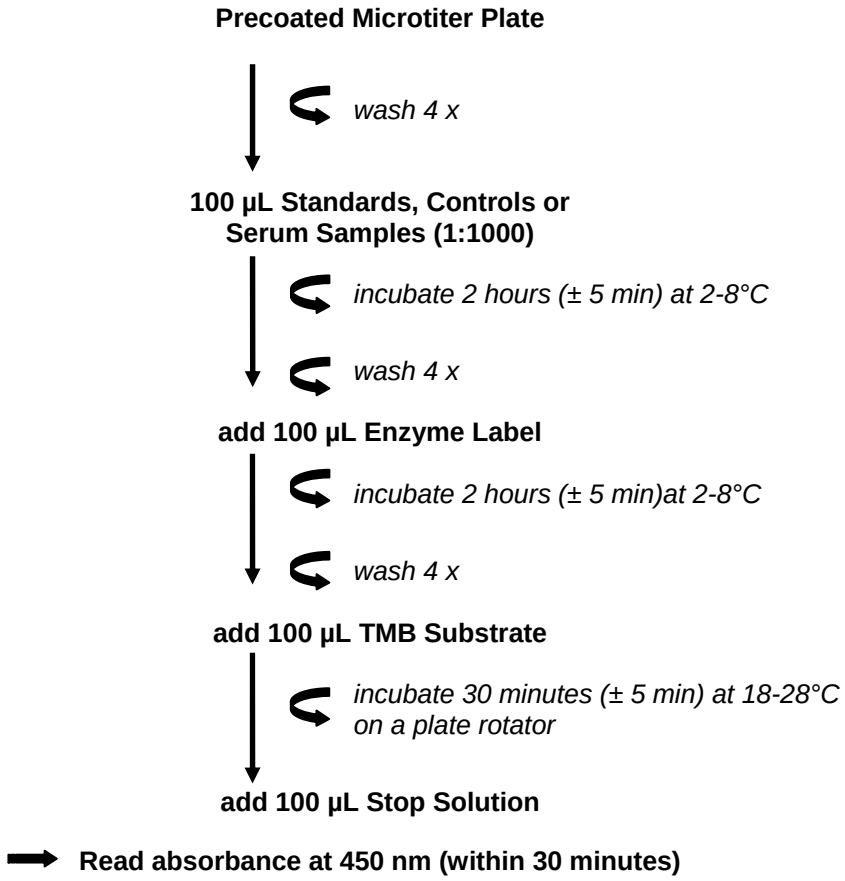

TIME TO RESULT: 4.5 HOURS

APPENDIX IV
SYMBOLS/SYMBOLE/ SYMBOLES/SIMBOLI/ SIMBOLOS

Symbol	Explanation
	Use By Verwendbar bis Utiliser jusqu'au Utilizzare entro Fecha de caducidad
REF	Catalogue number Bestellnummer Référence du catalogue Numero di catalogo Número de catálogo
LOT	Batch code Chargenbezeichnung Code du lot Codice del lotto Codigo de lote
IVD	*In Vitro* Diagnostic Medical Device *In Vitro* Diagnostikum Dispositif médical de diagnostic *in vitro* Dispositivo medico-diagnostico *in vitro* Producto sanitario para diagnóstico *in vitro*
	Contains sufficient for <n> tests Ausreichend für "n" Ansätze Contenu suffisant pour „n" tests Contenuto sufficiente per „n" saggi Contenudo sufficiente para <n> ensayos
	Consult Instructions for Use- Gebrauchsanweisung beachten Consulter le mode d'emploi Consultare le istruzioni per l'uso Consulte las instrucciones de uso
	Temperature limitation Zulässiger Temperaturbereich Limites de température Limiti di temperatura Limite de temperatura
BUF INC	Incubation Buffer Inkubations-Puffer Tampon d'incubation Tampone di incubazione Tampón de incubación

Symbol	Explanation
MP	Microtiter Plate Mikrotiter-Platte Microplaque Micropiastra Microplaca
BUF WASH 10X	Wash Buffer Concentrate (10x) Wasch-Puffer Konzentrat (10x) Tampon de lavage concentré (10x) Tampone di lavaggio concentrato (10x) Tampón de lavado concentrado (10x)
CAL A - CAL D	Calibrator A - D Kalibrator A - D Calibrateur A - D Calibratore A - D Calibrador A - D
CONTROL L	Low Control Kontrolle tief Contrôle bas Controllo basso Control bajo
CONTROL H	High Control Kontrolle hoch Contrôle elevé Controllo alto Control alto
EL IgM	Enzyme Label IgM Enzymmarker IgM Marqueur enzymatique IgM Marcato enzimatico IgM marcador enzimático IgM
SUBS TMB	TMB Substrate TMB Substrat Substrat TMB Substrato di TMB Substrato de TMB
SOLN STOP	Stop Solution Stopp-Lösung Solution stop Soluzione stoppante Solución de parada