anti-MAG ELISA

Procedure EK-MAG

A Commitment to Diagnostics

Pre-Analytics

Samples required:	Serum
Shipping samples:	Samples may be transported at RT (within 24 hrs). Avoid temperatures above 30°C.
Sample storage:	Samples may be stored for up to 1 year at -20°C. Store samples in aliquots.
Freeze/thaw-cycles:	Not recommended

Special Equipment

- There is no need for any special equipment.

Critical Steps

Samples and sample preparation

Samples:	Samples must not be hemolytic, lipemic or icteric
Sample preparation:	Dilute - vortex - equilibrate on ice for 30 minutes prior to using samples in the assay.

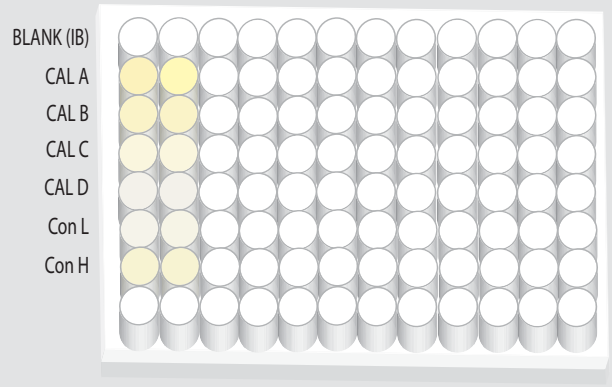
ELISA

Reagents	All reagents but TMB (substrate) must be used cold.
Washing steps:	Wash 4x with cold wash buffer prior to using it - empty wells completely.
	Set program to „plate mode" (serial dispensing followed by serial „aspiration") when using automated washer.
Incubations:	Both, first and second incubation step have to be performed at 2-8°C.
	Incubation with TMB substrate has to be performed at RT (18-28°C) - shaking on plate rotator.

Sample Preparation

Dilute samples 1:50 and put on ice for equilibration for 30 minutes.

Microtiter Plate Set-up



30 ´

ELISA

All reagents but TMB (substrate) must be used cold

Precoated Microtiter Plate

wash 4 x

add 100 µl of Controls and diluted Samples (1:50)

2 h — incubate 2 hrs (±5 min) at 2-8°C

wash 4 x

add 100 µl of Enzyme Label(s)

2 h — incubate 2 hrs (±5 min) at 2-8°C

wash 4 x

add 100 µl of TMB Substrate

30 ´ — incubate 30 (±5 min) at 18-28°C on a plate rotator

add 100 µl of Stop Solution

Read at 450 nm (within 30 min)

4.5 h

Time to Result: ~5 h

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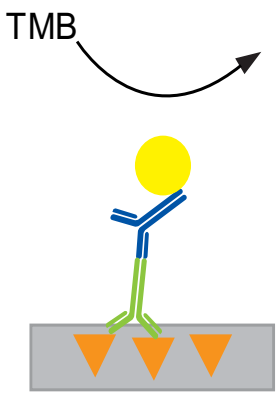
anti-MAG ELISA

Characteristics EK-MAG

A Commitment to Diagnostics

Intended Use

The anti-MAG ELISA test from BÜHLMANN is intended for the quantitative *in vitro* diagnostic determination of human IgM autoantibodies directed against Myelin Associated Glycoprotein (MAG).



Kit Formats

Code	Enzyme label	Wells
EK-MAG	IgM	1 x 96

Number of Tests in different Runs

Number of Runs	Number of Tests
1	41
2	34
3	27
4	20

Assay Performance Data

Intra-assay precision **6.5 %**

The intra-assay precision was calculated from the results of n = 20 pairs of values obtained in a single run (CV range: 2.1 - 10.3%).

Inter-assay precision **15.4 %**

The inter-assay precision was calculated from the results of n = 20 results of pairs of values obtained in 20 different runs.

Dilution linearity / Parallelism **147%**

Human serum samples with high titers of anti-MAG antibodies were diluted from 1:1000 down to 1:64000. The relatively high deviation in about 50% of samples is guessed to be due to antibody aggregation. Particularly as pathological sera exhibit highly elevated autoantibody titers discrimination between positive and negative sera is not affected.

Cut-off value **1000 BTU**

The cut-off value for anti-MAG antibodies was determined from samples from n = 150 asymptomatic volunteer blood donors from 18 - 70 years of age. The mean +3SD values resulted in a technical cut-off value of 729 BTU. For practical reasons we recommend to use a cut-off value of 1000 BTU.

Example of Results

EK-MAG (IgM)	absorbance (mean OD450)	BTU	% CV
Blank	0.040		
CAL D	0.085	1000	1.7
CaL C	0.312	3000	1.8
CAL B	1.076	15000	0.6
CAL A	2.199	70000	0.6
Crtl HIGH	1.178	17441	1.0
Crtl LOW	0.155	1550	1.8

Results

The absorbance measured is proportional to the titer of anti-MAG autoantibodies present in a given sample. The titers of the quantitative anti-MAG ELISA are expressed as Bühlmann Titer Units (BTU).

Standardisation

The Calibrators of the BÜHLMANN anti-MAG ELISA Kit were calibrated against an internal reference consisting of more than 10 human sera with low to high titers of anti-MAG antibodies.

Ordering code:
EK-MAG 96 wells (IgM)

BÜHLMANN

CE

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